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Investigation of the brain magnetisation transfer ratio, cognitive and neurological measures in prion disease

Thesis submitted for the degree of Doctor of Philosophy

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DECLARATION STATEMENT

I, Durr-E-Najaf Siddique, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Durr-E-Najaf Siddique

ABSTRACT

The work described in this thesis examines the application of magnetisation transfer ratio (MTR) measurement, a quantitative magnetic resonance imaging (MRI) technique, in evaluating patients with different forms of human prion disease. In particular whether MTR changes can be shown:

1. to correlate with clinical disease severity and disease type
2. to evolve on serial MRIs in clinically progressive disease

26 patients were assessed over 3 years. Global and regional cerebral MTRs were calculated using validated software and regions of interest manually defined on MTR maps. Whole brain, grey matter and white matter MTR histograms were computed and mean, peak height, peak location, and 25th, 50th and 75th percentile MTR histogram values were calculated to demonstrate localised and subtle diffuse pathological changes. A blinded assessment of DWI/FLAIR images was performed to determine MTR changes in areas with or without signal change on conventional MRI.

Patients were assessed using clinical video scores and neurological scales: Clinician's Global Impression of Disease Severity, Clinician's Dementia Rating, Alzheimer's disease Assessment Scale, Activities of Daily Living, Brief Psychiatric Rating Scale, Mini Mental Score Examination, Glasgow Coma Score and Rankin scores. Temporal changes in these tests of cognition, functional abilities, psychiatric symptoms and conscious state are described.

Spearman rank correlation and linear regression analyses were performed. At baseline, lower whole brain and grey matter MTR histogram parameters correlated significantly with lower cognitive, extrapyramidal and cerebellar impairment, as well as with MMSE, CDR, ADAS-COG, CGIS and Rankin scores. Longitudinal decline in multiple whole brain, white matter and grey matter MTR histogram parameters was associated with progressive extrapyramidal and CDR impairment. Four patients at baseline and 2 patients longitudinally had conventional MRI abnormalities.

Decreased MTR may be used as a biomarker of disease severity and is a potential outcome measure in future therapeutic trials in prion disease.

ABBREVIATIONS

AD	Alzheimer's Disease
ADL	Activities of Daily Living
ADAS-COG	Alzheimer's Disease Assessment Scale-Cognitive
ADC	Apparent Diffusion Coefficient
ANA	Anti-nuclear antibodies
AVMTR	Average MTR
BEHAVE-AD	Behavioural Pathology in AD scale
BET	Brain Extraction Tool
BPRS	Brief Psychiatric Rating Scale
BSE	Bovine spongiform encephalopathy
CDR	Clinician's Dementia Rating
CGIC	Clinician's Global Impression of Change
CGIS	Clinician's Global Impression of Severity
CI	95% Confidence Interval
CIBIC	Clinician's Interview-Based Impression of Change
CIS	Clinically Isolated Syndromes
CJD	Creutzfeldt-Jakob disease
CNC	Coma/Near-Coma Scale
CNS	Central Nervous System
CRS	Coma Recovery Scale
CSF	Cerebrospinal fluid
CT	Computerised Tomography
DRS	Disability Rating Scale
DTI	Diffusion Tensor Imaging
DV	Digital Video
DVD	Digital Video Disc
DWI	Diffusion-Weighted Imaging
EBV	Epstein-Barr virus
EEG	Electroencephalogram
Etc.	Et cetera
FAST	FMRIB's Automated Segmentation Tool
FFI	Fatal Familial Insomnia
FLAIR	Fluid attenuated inversion recovery MRI sequence
FLIRT	FMRIB's Linear Image Registration Tool
fMRI	Functional Magnetic Resonance Imaging
FMRIB	Oxford centre for Functional Magnetic Resonance Imaging of the Brain
FOV	Field of View
FSL	FMRIB Software Library
FTP	File Transfer Protocol
FU	Follow-up
GA	General Anaesthesia
GBS	Gottfries-Brane-Steen Test
GCS	Glasgow Coma Score
GDS	Global Deterioration Scale
GE	General Electric
GIC	Global Impression of Change
GP	General Practitioner
GSS	Gerstmann-Sträussler-Scheinker disease
HD	Huntington Disease
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
IADL	Instrumental Activities of Daily Living

IB	Immunoblotting
ICM	Information-Concentration-Memory Test
IDDD	Interview for Deterioration in Daily living activities in Dementia
IHC	Immunohistochemistry
InhPrD	Inherited Prion Disease
LCFS	Level of Cognitive Functioning Scale
M_0	Absence of saturation
M_S	Presence of saturation
MCI	Mild Cognitive Impairment
MM	Codon 129 Methionine homozygous
MMSE	Mini Mental State Examination
MRC	Medical Research Council
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MREC	Multi Regional Ethics Committee
MS	Multiple Sclerosis
MT	Magnetisation Transfer
MTI	Magnetisation Transfer Imaging
MTR	Magnetisation Transfer Ratio
MTR25%	Magnetisation Transfer Ratio at 25^{th} percentile
MTR50%	Magnetisation Transfer Ratio at 50^{th} percentile
MTR75%	Magnetisation Transfer Ratio at 75^{th} percentile
MV	Codon 129 Methionine/Valine heterozygous
NAA	N-acetylaspartate
NABT	Normal Appearing Brain Tissue
NAGM	Normal Appearing Grey Matter
NAWM	Normal Appearing White Matter
NCJDSU	National CJD Surveillance Unit
NEX	Number of Excitations
NMR	Nuclear Magnetic Resonance
No.	Number
NPC	National Prion Clinic
NPI	Neuropsychiatry Inventory
NHNN	National Hospital for Neurology and Neurosurgery
OPRI	Octapeptide Repeat Insertion
PACS	Picture Archiving and Communication Systems
PD	Parkinson's Disease
PD-weighted	Proton Density-weighted
PET	Positron Emission Tomography
PH	Peak Height
PL	Peak Location
PPMS	Primary Progressive Multiple Sclerosis
PRNP	Human prion protein gene
PrP	Prion Protein
PrP^c	Normal Prion Protein
PrP^{Sc}	Abnormal Prion Protein
PSMS	Physical Self-Maintenance Scale
PSP	Progressive Supranuclear Palsy
pu	Percentage units
RF	Radio Frequency
RRMS	Relapsing Remitting Multiple Sclerosis
ROI	Region of Interest
SAP	Severely Affected Protocol of PRION-1 video
SCAG	Sandoz Clinical Assessment Geriatric

sCJD	Sporadic Creutzfeldt-Jakob disease
SD	Standard deviation from the mean
SE	Standard error
SIB	Severe Impairment Battery
SKT	Syndrom Kurztest
SOP	Standard Operating Procedure
SPECT	Single Photon Emission Computerised Tomography
SPMS	Secondary Progressive Multiple Sclerosis
T1/T2WI	T1 or T2-weighted MRI Imaging
TFC	Total Functional Capacity score
TE	Echo time
TENS	Transcutaneous Electrical Nerve Stimulation
TR	Repetition Time
TSE	Transmissible Spongiform Encephalopathy
UK	United Kingdom
USA	United States of America
μT	micro-Tesla
vCJD	Variant Creutzfeldt-Jakob disease
VDRL	Venereal Disease Research Laboratory test (syphilis)
VV	Codon 129 Valine homozygous
WHO	World Health Organisation

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AIMS

The aims of the work described in this thesis were, in patients with prion disease, to:

a) Assess the associations between brain MTR and clinical scores at baseline

b) Quantify decline in cerebral MTR and clinical scores longitudinally

c) Quantify abnormalities in MTR in patients with and without signal change on conventional MRI

d) Determine the range and pattern of tests of cognition, motor performance, functional abilities, psychiatric symptoms, general health and conscious state and describe their temporal changes

in order to evaluate change in MTR as a potential objective outcome measure in treatment trials in prion disease

HYPOTHESES

a) MTR changes will be demonstrated in normal appearing brain tissue, facilitating an early diagnosis in prion disease

b) Longitudinal serial MTR changes can be used to monitor disease progression, as a quantitative biomarker of disease severity in prion disease

INVESTIGATION OF THE BRAIN MAGNETISATION TRANSFER RATIO, COGNITIVE AND NEUROLOGICAL MEASURES IN PRION DISEASE

1. INTRODUCTION

Magnetisation Transfer (MT) is a quantitative magnetic resonance imaging (MRI) technique, which has been shown to be sensitive to pathological change in the central nervous system (CNS) in diseases such as Alzheimer's disease (AD) [1,2] and Multiple Sclerosis (MS) [3,4]. To assess the sensitivity of MT to pathological change in the brain due to prion disease, Magnetisation Transfer Ratios (MTRs) were measured within and outside areas of signal change on conventional MRI diffusion-weighted imaging (DWI) and fluid attenuated inversion recovery (FLAIR) images, and correlated with clinical markers of disease progression. This first investigation of MT in prion disease, performed in patients being followed up as part of the PRION-1 Trial [5], was intended to determine whether MT may be useful in both establishing the diagnosis of human prion disease at an early stage when conventional MR abnormalities may be absent, and in monitoring disease progression.

1.1 HUMAN PRION DISEASES

Human prion diseases, also known as transmissible spongiform encephalopathies (TSEs) have been traditionally classified into Creutzfeldt-Jakob Disease (CJD), Gerstmann-Straussler syndrome (GSS) and Kuru [6,7]. They are progressive, uniformly fatal and rare neurodegenerative disorders [7], and the transmissible agent, the prion, consists of an abnormal isoform of prion protein (PrP) called PrP^{sc}, derived from the normal cellular isoform, PrP^{c}, by a post-translational mechanism [8]. The

histopathological appearances are characterised by spongiform change, neuronal loss, reactive astrocytosis and the presence of PrP amyloid plaques [9].

Etiologically, human prion diseases are subdivided into sporadic, inherited and acquired forms (Table 1) [7, 8], with CJD, GSS and Kuru now considered as clinicopathological syndromes within a wider spectrum of the disease.

Table 1: Human prion diseases

Type	Clinical syndromes	Etiology
Acquired CJD	Kuru	Exposure to human prions during cannibalistic feasts
	Iatrogenic CJD	Accidental inoculation with human prions
	Variant CJD (vCJD)	Exposure to Bovine spongiform encephalopathy (BSE)-like prion strain
Sporadic CJD (sCJD)	sCJD	Unknown, possible somatic PRNP (human prion protein gene) mutation or spontaneous conversion of PrP^{c} to PrP^{sc}
Inherited	Familial CJD, GSS, Fatal familial insomnia (FFI)	Germline PRNP mutation

1.1.1 Sporadic prion disease

sCJD has an incidence of 1 to 2 per million in the worldwide population, accounting for 85% of the incidence of human prion disease, and its aetiology is unknown. The age of onset is usually between 45 to 75 years, and clinical progression is typically over weeks, with 70% of patients dying in less than 6 months [7]. The clinical phenotype of sCJD is typically one of rapidly progressive dementia and multifocal neurological features which include myoclonus, ataxia, pyramidal and extrapyramidal signs. Atypical cases are a particular feature of this form of CJD. The host prion protein (PRNP) codon 129 genotype (where either of the polymorphic alleles methionine or valine is expressed) and the molecular strain of the transmissible prion agent affect the phenotype [10]. Progression of the disease causes loss of mobility and independence, leading to akinetic mutism and eventually death.

The World Health Organisation has published criteria for the classification of sCJD (www.advisorybodies.doh.gov.uk/acdp/tseguidance/tseguidance_annexb.pdf):

I Rapidly progressive dementia

II A Myoclonus

B Visual or cerebellar problem

C Pyramidal or extrapyramidal features

D Akinetic mutism

III A Typical electroencephalogram (EEG) (Figure 1)

B Positive CSF 14-3-3

Possible CJD: I and 2 of II and duration less than 2 years

Probable CJD: I and 2 of II and IIIA or possible CJD and IIIB

Definite CJD: Neuropathologically confirmed diagnosis

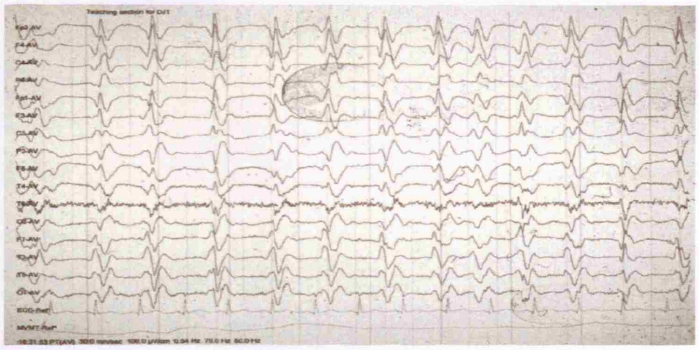


Figure 1: EEG showing periodic sharp wave complexes in sCJD

The main differential diagnoses include paraneoplastic disease, Alzheimer's disease, other forms of CJD, dementia with Lewy bodies, encephalitis and vascular dementia. Patients with a rapidly progressive dementia and focal neurological signs should have sCJD as their primary differential diagnosis. Presence of cerebellar, visual and pyramidal signs favour a diagnosis of sCJD over other dementias and duration of symptoms is important [11].

Visual disturbance, exemplified by the Heidenhain variant of sCJD [12] is more common than auditory disturbance. Visual symptoms have been reported as presenting symptoms of sCJD [12, 13], as have abnormal eye movements [14]. An isolated field defect or visual loss in the absence of other MRI, EEG or laboratory abnormalities may occur. In Will et al [15], of 137 sCJD cases, 9% presented with visual disturbance and thirteen percent demonstrated cortical blindness during the illness. Cortical deafness has been reported as a presenting feature of sCJD [16]. Hearing impairment is however unusual in sCJD; there were no reports of auditory symptoms in the cases reported by Will. Symptoms may be lateralised to left hemisphere (right sided posturing, sensory disturbance and aphasia), right hemisphere (left sided neglect and dressing apraxia) or occipital lobe [13]. These localising symptoms may be matched by EEG and MRI changes.

This variation in clinical presentation is not fully explained but is influenced by the characteristics of the host prion protein gene (PRNP) and pathological prion strain type. The PRNP codon 129 genotype is either MM (methionine homozygous), MV (methionine/valine heterozygous), or VV (valine homozygous). Molecular strain typing is based on the biological properties of the abnormal prion protein as determined by prion protein (PrP) fragment size following partial protease digestion and the pattern of PrP glycosylation. Codon 129 methionine and valine homozygosity have been reported as predisposing factors for sCJD [17, 18, 19].

Several human prion protein types have been identified that are associated with different phenotypes of CJD [20, 21]. In sCJD, Collinge [20] showed two strains (types 1 and 2), with MM patients having both types 1 and 2, whereas MV and VV cases had only type 2. Parchi found type 1 only in MM patients and type 2 in MM, MV and VV patients. Three distinct groups are described by Tranchant et al [17] in their fourteen

patients with sCJD. The first group (10 patients) were MM and strain 1; initial signs were cognitive or visual, followed by dementia and myoclonus. The second group (3 patients) were VV and strain 2; clinically they showed cerebellar ataxia and later dementia. The third group (1 patient) was MV and type 1; cerebellar ataxia and dementia appeared simultaneously. These findings are similar to that of Parchi [21] who reported 19 sCJD cases: the 13 MM patients presented with early dementia, the 3 VV patients with ataxia and the MV patients with ataxia and dementia. In Parchi's 1999 paper [22] there was further delineation of sCJD types. MM1 (67%) presented with early cognitive disturbance, ataxia, myoclonus and a typical EEG; VV2 (16%) presented with ataxia and only occasionally a typical EEG pattern; MV2 (9%) had a longer disease duration, extrapyramidal signs and only occasionally a typical EEG pattern. In Parchi's 2000 paper [23] VV1 was described as occurring in younger patients with a longer disease duration and late dementia.

Whether strain and genotype are independent or linked factors in determining clinical phenotype is yet to be fully resolved.

1.1.2 Variant prion disease

Human variant prion disease (vCJD), caused by exposure to a BSE-like prion strain[24], was first described in 1996 [25] as a new form of CJD affecting young patients who had a psychiatric presentation and a slower rate of clinical progression. The transmissible prion agent has a characteristic strain type on Western blotting termed type 4 PrP^{sc} [20] which differs from the three types (1-3 PrP^{sc}) found in other types of human prion disease. By December 2007, 166 definite or probable vCJD cases had been reported in the UK (http://www.cjd.ed.ac.uk/figures.htm), as well as cases in France [26, 27], USA [28], Ireland [29], Italy [30] and Canada [31]. Opinions differ on the future course of the

primary vCJD epidemic in the UK [32, 33]. Cases caused by exposure to BSE are still occurring. Transfusion related vCJD and iatrogenic exposure are now recognised risk factors in terms of future cases and potential for transmission [34].Risk factors for the development of vCJD include age and residence in the UK. By December 2006 all reported cases examined had demonstrated methionine homozygosity at codon 129 of the prion protein gene and where molecular typing was carried out the same strain (type 4 PrP^{sc}) was demonstrated in all patients. Department of Health Diagnostic Criteria for variant CJD are shown below:

I A) Progressive neuropsychiatric disorder

B) Duration of illness > 6 months

C) Routine investigations do not suggest an alternative diagnosis

D) No history of potential iatrogenic exposure

II A) Early psychiatric symptoms *

B) Persistent painful sensory symptoms **

C) Ataxia

D) Myoclonus or chorea or dystonia

E) Dementia

III A) EEG does not show the typical appearance of sporadic CJD*** (or no EEG performed)

B) Bilateral pulvinar high signal on MRI scan

IV A) Positive tonsil biopsy

Definite: IA (progressive neuropsychiatric disorder) and neuropathological confirmation of vCJD ****

Probable: I and 4/5 of II and III A and III B or I and IV A

Possible: I and 4/5 of II and III A

* depression, anxiety, apathy, withdrawal, delusions

** includes both frank pain and/or unpleasant dysaesthesia

*** generalised triphasic periodic complexes at approximately one per second

****spongiform change and extensive PrP deposition with florid plaques, throughout the cerebrum and cerebellum.

vCJD is characterised by behavioural, psychiatric or sensory disturbances (including painful dysaesthesias in limbs) at presentation [25, 35, 36]. vCJD is usually seen in young adults, but age at onset may vary between 12 to 74 years, and the clinical course is also prolonged (9-35 months, median=14 months) [36]. With disease progression, a cerebellar syndrome with myoclonus, chorea and athetosis may develop. Dementia develops late, with progression to akinetic mutism and eventually death in all cases. Difficulty is encountered in initial diagnosis due to the early psychiatric and behavioural symptoms. In Will's description of the psychiatric features of 33 early vCJD patients [37], seventeen were seen first by a psychiatrist and all but one demonstrated psychiatric symptoms in the early stages of the disease. These included depression, anxiety, withdrawal, first rank symptoms, aggression and hallucinations. Sensory symptoms associated with vCJD are not commonly seen in other types of prion disease [38]. In the first 50 vCJD cases, 10 had sensory symptoms from the outset; 32 had persistent sensory symptoms, 9 had possible sensory symptoms at some time that did not persist, and 8 had no sensory symptoms at all. Symptoms included limb pain (63% of patients and usually lower limbs), paraesthesia (31%), dysaesthesia (28%), numbness (25%) and cold feelings (25%) [39]. Sensory symptoms were treated variously with non-steroidal anti-inflammatory drugs, simple analgesics, carbamazepine, antidepressants and transcutaneous electrical nerve stimulation (TENS), though few were effective.

Movement disorders become apparent evolving into chorea, dystonia and myoclonus. As deterioration occurs, limb rigidity and primitive reflexes become evident, swallowing becomes difficult, the patient becomes fully dependent on carers for everyday tasks and becomes bed bound before death. There is currently no simple diagnostic blood test for vCJD. 14-3-3 analysis of cerebrospinal fluid does not differentiate between sCJD and vCJD and the EEG does not show the characteristic periodic sharp wave complexes of sCJD. In the appropriate clinical setting, the pulvinar sign on MRI [40] is an extremely helpful radiological sign. Uniquely, in vCJD, the abnormal prion protein, type 4 PrP^{sc}, is found in the lymphoreticular system (Figure 2), outside the central nervous system.

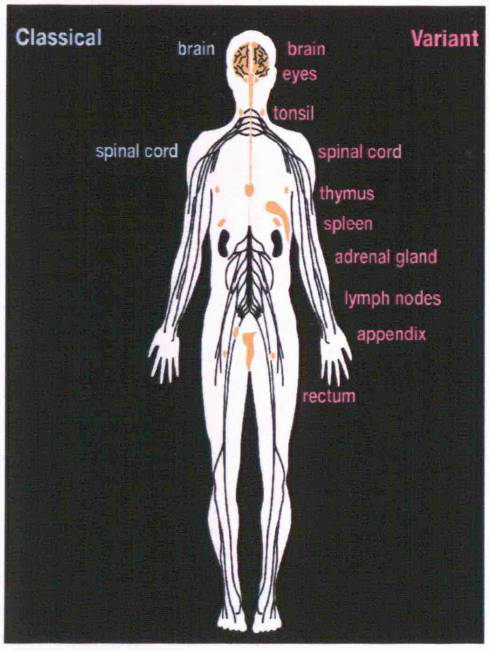


Figure 2: Distribution of PrP^{sc} in human tissues in vCJD and classical CJD

In the clinical setting of vCJD a tonsil biopsy may therefore be used to achieve a definitive diagnosis (currently 100% specific at the National Prion Clinic, unpublished data) thus avoiding a brain biopsy [41].Molecular typing of the biopsy tissue reveals the same strain in all vCJD patients. Differential diagnoses include

paraneoplastic syndrome, familial and early onset dementias and metabolic syndromes.

Initial treatment is tailored to the psychiatric presentation, managed with the help of anti-depressants and antipsychotics. Symptomatic treatments are used as the disease progresses. Experimental therapies used by some patients include Pentosan Polysulphate, an anticoagulant administered intraventricularly via a pump or quinacrine, given orally.

1.1.3 Inherited prion disease

Inherited prion disease (InhPrD), transmitted in an autosomal dominant manner, is caused by point or insertional mutations within the PRNP gene on chromosome 20 (Figure 3). It accounts for 10-15% of the incidence of human prion disease [18, 42]. WHO diagnostic criteria exist as in sporadic and variant prion disease [43].

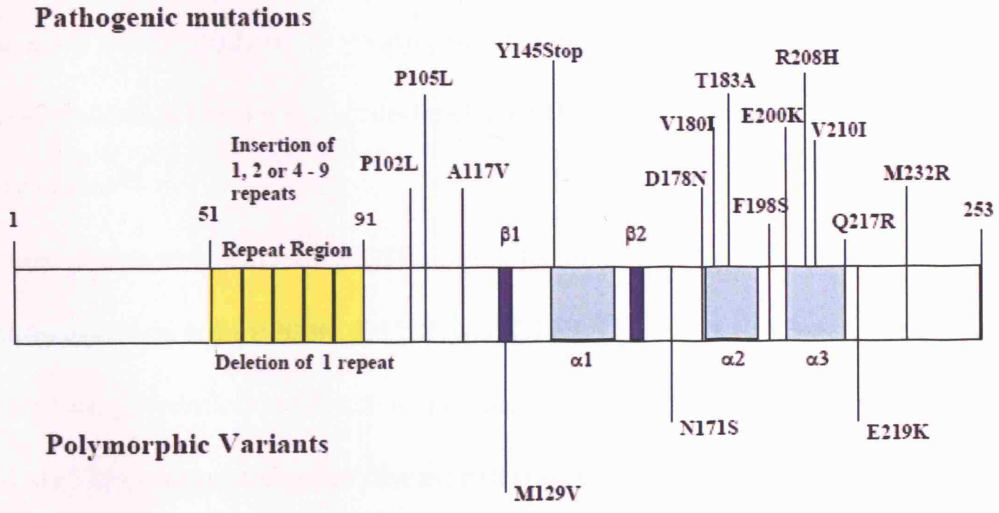


Figure 3: Pathogenic mutations and polymorphic variants in the prion protein gene

The clinical phenotype has historically been subdivided into Gerstmann-Straussler-

Scheinker syndrome (GSS), Fatal Familial Insomnia (FFI) and Familial CJD. The principle clinical features in GSS are a slowly progressive ataxia with later onset Dementia while FFI is characterised by insomnia, autonomic dysfunction, motor signs and hallucinations. Familial CJD presents with a rapidly progressive dementia, myoclonus and pseudoperiodic discharges on EEG [44].

These clinical descriptions preceded the advent of molecular genetic diagnosis. As clinical experience of patients with different genetic mutations has grown it has become apparent that there is considerable clinical overlap and heterogeneity of phenotypes even between individuals within the same family and with the same mutation.

There are several hurdles for clinicians in diagnosing inherited prion disease. The clinical phenotype in inherited prion disease often overlaps with other more common causes of dementia such as Alzheimer's disease. Bruton et al [45] describe an autopsy study where 40% of neuropathologically confirmed prion cases were undiagnosed while alive. In one study of 36 young patients with early onset dementia, screened for a genetic mutation Finckh et al found that PRNP muations accounted for 4 out of 12 positive cases[46].

The most common worldwide PRNP mutations are P102L (proline to leucine mutation at codon 102) E200K, D178N and OPRI [44]. Several European studies have reviewed the population incidence of inherited prion disease. Windl [18] in Germany found 40/578 in suspected prion disease referrals to their surveillance unit, (13/40 D178N, 8/40 E200K, 7/40 P102L, 5/40 OPRI). Laplanche [19] in France found 8/57 (all E200K). Pocchiari [47] in Italy found 6/38 D178N, 2/38 P102L and 30/38 with E200K or V210I. Kovanen [48] in Finland found 12/44 with D178N. In the UK, the 6 OPRI mutation is the most frequently detected [44].

Inherited prion disease is the most easily diagnosed of all types of prion disease, as a blood test alone is the definitive test, rather than brain biopsy in sCJD or tonsil biopsy in vCJD.

Most mutations co-segregate with methionine at codon 129. Of 455 cases identified by the EUROCJD collaborative surveillance project (including Australia, Austria, Canada, France, Germany, Italy, Holland, Slovakia, Spain, Switzerland and the UK), the majority of cases were either MM 67.9% (vs 39% in controls) or MV 25.8% (50% in controls), with few valine homozygotes 6.3% (vs 11% in controls) [49].

In inherited prion disease as a whole there is a strong negative correlation between age of onset and duration of disease [44], possibly due to inability of the aging brain to degrade pathological prion protein.

The main differential diagnosis once prion disease is suspected in the older patient is sCJD, highlighting the importance of genetic screening for mutations in these patients. Although the incidence of sCJD is similar in most countries [50], incidence of PRNP mutations shows large variability from the 15% seen worldwide. 47% of patients with inherited prion disease may have no reported family history [51], making differentiation from sCJD difficult. Factors such as non-paternity, lack of knowledge of family tree and death prior to disease onset are potential explanations, but the possibility that some mutations are occurring de novo [52] or that the mutations are not fully penetrant [53] exists.

1.1.3.1 E200K

E200K is the most common inherited prion disease worldwide. Patients with this mutation have very similar clinical features to sCJD. There is a rapidly progressive dementia with myoclonus and pyramidal, extrapyramidal or cerebellar signs. Also,

like sCJD, neuropathology shows absence of PrP plaques. Heterozygous, and a small number of homozygous individuals with an earlier age of onset occur (median age 50 instead of 58), both having similar phenotypes. Median duration of illness is the shortest of all inherited prion disease at 5 months. Triggers for disease onset have been studied in E200K. Psychological stress (divorce, death of a close relative, retirement, loss of job), surgery with prolonged anaesthesia, serious accidents or current infectious disease have been noted prior to disease onset[53, 54].

1.1.3.2 OPRI

The insertion of more than three octapeptide repeats causes inherited CJD. 6 OPRI was the first PRNP mutation described in the literature in 1989 [55]. A pedigree of over 80 affected and 100 at risk individuals has been traced from a common ancestor in the UK [44]. Clinical features include a cortical dementia with apraxia, cerebellar ataxia and pyramidal and extrapyramidal signs. A premorbid personality disorder of aggression and delinquency has been described [56]. Age of onset varies from 3rd to 6th decade with variable rates of disease progression. Croes et al [57] noted a negative correlation between age of onset and number of insertions, fewer repeats showing later onset and shorter disease duration.

1.1.3.3 P102L

P102L mutation is a typical example of GSS syndrome, manifesting as a slowly progressive ataxia with later pyramidal features and dementia. Phenotypic variability is common, and cases with dementia in the absence of prominent ataxia have been described, with a median disease course of 4 years [58].

1.1.3.4 D178N

Patients with the D178N mutation develop insomnia, autonomic dysfunction and myoclonus at a median age of 50 with a short median disease course of 11 months. This clinical syndrome, coupled with selective degeneration of the anteroventral and dorsomedial thalamus is termed FFI. Goldfarb [59] described this mutation on an 129M chromosome leading to FFI and on an 129V chromosome leading to familial CJD. This differentiation does not hold true in all families, and other PRNP mutations been described in association with similar sleep and autonomic disturbance.

A future hope for patients with inherited prion disease, and their descendants is that they may benefit from novel therapies that prevent abnormal prion protein propagation and which might be given prior to clinical disease onset.

1.1.4 Iatrogenic prion disease

Iatrogenic prion disease is caused largely by infection transmitted from cadaveric human growth hormone prior to 1985, dura mater grafts manufactured and distributed before the mid-1980s, corneal grafts or blood transfusion. Patients iatrogenically infected with vCJD via blood transfusion represent a new mode of transmission of iatrogenic disease, as discussed earlier. The pathogenesis of vCJD differs from other forms of human prion disease in that there is significant systemic involvement of lymphoreticular tissue. This has raised concerns that infection might be transmitted via contaminated surgical instruments.

The Department of Health and Health Protection Agency recently reviewed the risks of transmission occurring under these circumstances (www.hpa.org.uk/infections/topics_az/cjd/information_documents.htm). The infection risk was modelled for those patients coming into contact with surgical instruments which were used on a patient with prion disease. Risk was deemed highest if the

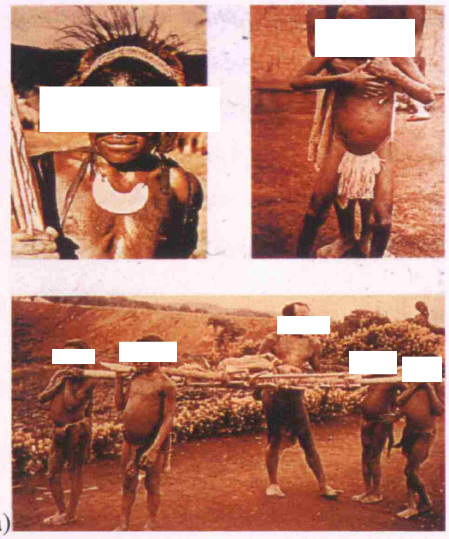
instruments had only been used and decontaminated a small number of times since being used on a patient with prion disease. Factors considered in whether informing exposed patients of the potential risk included the clinical condition of the patient with prion disease, the infectivity levels of the tissues the instruments had been used on, the type of instruments used, the decontamination processes in place and whether the instruments could be traced. The CJD Incidents panel advise contacting patients who are at a one percent risk of being infected with prion disease via this route, as determined by the risk model described. These patients are advised not to donate blood, organs or tissues, to inform practitioners if surgical, dental or endoscopic procedures are planned and to advise next of kin in case of emergency medical treatment being required.

Clinical features in iatrogenic prion disease depend on the route of inoculation. In peripherally inoculated cases, cerebellar presentation rather than dementia predominates, and dementia is not invariably present. Apart from vCJD acquired from blood transfusion, cases are otherwise clinically similar to sCJD with ataxia, myoclonus, rigidity and akinetic mutism. Incubation periods range from 18 months to up to 30 years, with a mean period of 3 years for centrally acquired and 12 years for peripherally acquired disease [60].

1.1.5 Kuru

This acquired form of prion disease was discovered in the 1950s when an epidemic of a neurodegenerative disease characterized by a progressive ataxia was found among the Fore linguistic group of the Eastern Highlands of Papua New Guinea (Figures 4 a and b). Field work by several groups suggested that kuru was transmitted during cannibalistic feasts [61, 62, 63, 64].

Clinical features include a cerebellar syndrome with truncal ataxia and tremor that progresses through defined clinical stages. Cognition can remain unaffected initially, but all patients then develop progressive dementia and death.



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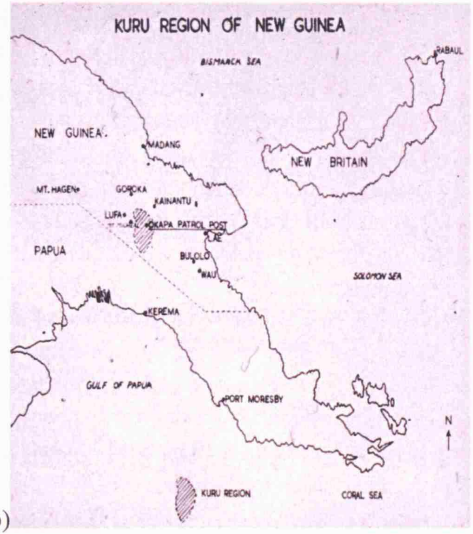


b)

Figure 4: Kuru in the 1950s (a) in the Eastern Highlands of Papua New Guinea (b)

The first cases were identified in the 1920s, and the peak of the epidemic was in the 1950s, mostly among women and children who participated in mortuary feasts. Patients with kuru are currently alive [65], being exposed before the cessation of cannibalism in the late 1950s. Incubation periods have been estimated between 5 and 56 years; incubation time and resistance to prion disease are increased in codon 129 heterozygotes. Being the only previous example of dietary exposure to prions in humans before variant CJD, this group of patients has provided useful insights into human prion disease.

1.2 MANAGEMENT OF HUMAN PRION DISEASES

1.2.1 Referral

In the United Kingdom, patients come to the attention of specialists in prion disease through a chain of referral. Patients usually present to their General Practitioner who

refers to the local neurologist. A national referral agreement is currently in place so all patients with a suspected diagnosis of prion disease are referred jointly to the National Prion Clinic (NPC), National Hospital for Neurology and Neurosurgery, London, and the National CJD Surveillance Unit (NCJDSU), Western General Hospital, Edinburgh, with notification to the Health Protection Agency.

1.2.2 Investigation and Diagnosis

At first contact with a prion disease specialist the patient will usually have a history suggestive of one of the 4 types of prion disease.

Variant patients are usually under 40, residents of the UK for several years in the 1980s and 1990s with subtle psychiatric symptoms, pain or dysaesthesia in limbs, a movement disorder (either myoclonic or cerebellar) or signs of early cognitive deficits, particularly episodic memory problems. A new cohort of patients has been identified recently [34] who were exposed to a potentially infected blood transfusion from a donor who later developed prion disease. This cohort present in the same way, but have a wider range of age of onset depending on when the transfusion was received.

Patients with inherited prion disease sometimes have a known family history of a specific mutation in the prion protein gene, or more usually a history of ataxia or an early onset dementia in first degree relatives. At risk individuals may approach prion disease specialists for information on the disease, to help understand the disease and care for ill relatives, for information prior to starting a family, or for presymptomatic testing and genetic counselling. Other patients present with early symptoms which vary according to the patient's mutation. Examples include patients with early cognitive deficits associated with a 6 octapeptide repeat insertion (6 OPRI), or

cerebellar symptoms with the PRNP P102L patients.

Iatrogenic patients have a history of ocular or neurosurgery, growth hormone supplementation or infected blood transfusion. In peripherally inoculated cases, cerebellar presentation rather than dementia predominates.

Sporadic patients tend to be the oldest patients and present at the most advanced stages of disease, due to the rapid disease progression These patients are often bedbound and mute at time of referral, or may pass away in the few days between referral and first patient visit.

Since prion disease is rare, it is important to rule out other causes of dementia. Any outstanding tests not performed by the referring hospital are completed. These include anti-nuclear antibodies (ANA), syphilis (VDRL), HIV, EBV and HSV. If there is any suggestion of a paraneoplastic syndrome a whole body CT or PET is performed. Neuropsychometry looking for types and patterns of cognitive impairment is often performed in patients who are well enough to participate.

More specific tests to confirm prion disease are described in Table 2 [8]:

Table 2: Diagnosis of human prion diseases

Sporadic (classical) CJD	• Serial EEG shows pseudoperiodic complexes in most cases but often at an advanced clinical stage • CSF 14-3-3 protein usually positive • CT and MRI normal, or atrophy, abnormal signal in basal ganglia on T2 weighted/DWI/FLAIR sequences, or cortical ribboning • PRNP analysis: no pathogenic mutations, most are 129 MM (VV and MV may be of longer duration, clinically atypical, and with EEG less often positive) • Brain biopsy in highly selected cases (to exclude treatable alternative diagnoses): Prion protein immunocytochemistry or western blot for PrP^{Sc}
Iatrogenic CJD	• EEG, CSF, and MRI generally less helpful than in sporadic cases • PRNP analysis: no pathogenic mutations, most are 129 homozygotes • Brain biopsy in highly selected cases (to exclude treatable alternative diagnoses): Prion protein immunocytochemistry or western blot for PrP^{Sc}
vCJD (Human BSE)	• EEG: non-specific slow waves • CSF 14-3-3 may be elevated or normal • MRI: most show high signal in posterior thalamus

	bilaterally on T2 weighted/DWI/FLAIR sequences but may be normal in early clinical diagnosis • PRNP analysis: no mutations, all 129 MM to date • Tonsil biopsy: characteristic prion protein immunostaining and PrP^{Sc} on western blot (type 4t)
Inherited prion disease (IPD)	• PRNP analysis: diagnostic, codon 129 genotype may predict age at onset in pre-symptomatic testing

Once the diagnosis of prion disease is confirmed the patient and/or their next of kin are informed by a senior member of the specialist prion team. At the National Prion Clinic (NPC) a specialist nurse and a counsellor are usually present. As well as discussing the diagnosis, written information is given to the family to take home and a management plan instituted. Advice available for clinicians caring for those with prion disease has been recently reviewed by the Health Protection Agency in October 2006 and can be found at www.hpa.org.uk/infections/topics_az/cjd/information_documents.htm.

1.2.3 Management

Prion disease creates its own unique management challenges and difficulties when keeping up with the needs of a rapidly deteriorating patient. Early involvement from the relevant multidisciplinary team is important, usually comprising a social worker, community psychiatric nurse, home carers, GP, specialist prion doctors, counsellor, patient's family or friends and specialist prion nurse. The multidisciplinary team may become very large, so careful case coordination and communication is required to ensure its efficiency. Conflict between family members or patient and family can occur, and may prove to be a challenge while managing a fatal disease.

In terms of structure of care, palliative care for rapidly degenerative conditions needs to be considered. The practicalities of home care versus hospice care are considered in each individual situation. Adaptations for the home and movement aids are important,

particularly because patients are often initially nursed at home. The proximity of a suitable hospice placement, at relatively short notice, is often challenging. Practical problems of finance, transport and care provision are common.

All aspects of patient care in this multifaceted disease need to be considered. Basic care of pressure areas, bowels and prevention of falls is mandatory. As mobility declines appropriate aids may become necessary. Movement disorders can be helped with clonazepam for myoclonus, baclofen as an antispasmodic or olanzapine, which may help with hallucinations or delusions, as well as movement disorders. Bulbar function often becomes affected, requiring careful swallowing and nutritional assessment. Deciding whether and when to place a nasogastric tube or gastrostomy tube (radiologically guided) for feeding is difficult for patient, family and clinician. Pain and sensory symptoms can be relieved with gabapentin or amitriptyline; anti-depressants or antipsychotics may prove beneficial as altered mood and behaviour may be a prominent feature.

As communication difficulties increase with disease progression there may be frustration on the part of both patient and family. This creates short-term practical problems and longer term consent problems. Some patients choose to write a Living Will, or other document stating their wishes for the time when they are unable to make or communicate decisions. The difficult issue of consent for post mortem examination is raised at an appropriate point with the patient and/or family. Concerns are addressed by patients and their partners about the possibility of horizontal transmission between sexual partners, or vertical transmission during childbirth or breastfeeding. There is little evidence to support transmission via these routes and this is referred to in the Health Protection Agency's advice above. Different problems arise in mutation positive younger patients who are uncertain about how or when the

disease might affect them. This group of patients is constrained by being unable to donate blood or other tissues such as cornea or dura mater, and having to notify dentists and surgeons of their status to prevent iatrogenic transmission of disease. This group may benefit the most from future therapies that prevent prion propagation since such treatment could conceivably be commenced prior to clinical disease.

Stigma and ignorance of prion disease is still a problem within the health system and the public. Heightened media interest means patient confidentiality requires vigilance. The distress caused to patients and families as a consequence of the nature and origin of the disease should be anticipated and managed well. Misleading or conflicting information obtained from the internet or media may cause confusion.

The National Prion Clinic and the National CJD Surveillance Unit also work in an advisory role giving help to patients, carers and families. They, along with the Department of Health, provide access to the National CJD Care Package, a fund used to assist with the care of all patients with prion disease. It covers the cost of services which cannot be met by local health authorities. Patient support groups exist, including the CJD Support Network and the Human BSE Foundation.

Management of a rapidly progressive, invariably fatal disease under intense media scrutiny may be difficult. Effectively managed, appropriate and timely care is required to ensure that each patient's needs are met.

1.3 NEUROPATHOLOGICAL CHANGES IN HUMAN PRION DISEASES

Macroscopic examination of the brain may not reveal any abnormality in CJD [66]. Cerebral atrophy, either diffuse or focal, is a characteristic finding, and may preferentially involve the occipital lobe, striatum, thalamus or cerebellum [9]. Severe

thalamic atrophy is a characteristic feature of FFI, and cerebellar atrophy is prominent in Kuru, GSS and iatrogenic CJD in human pituitary hormone recipients. Histopathological examination of the central nervous system provides a definitive diagnosis in all forms of human prion disease [67], with prion diseases characterised by spongiform change, neuronal loss, reactive astrocytosis and PrP amyloid plaques. These changes are incorporated in the WHO neuropathological criteria for CJD and other human prion diseases (Table 3) [9].

Table 3: Neuropathological criteria for CJD and other human prion diseases

Creutzfeldt-Jakob disease (CJD)

A) Sporadic, iatrogenic (recognised risk) or familial (same disease in first degree relative or disease-associated PRNP mutation):
- Spongiform encephalopathy in cerebral and/or cerebellar cortex and/or subcortical grey matter; and/or
- Encephalopathy with prion protein immunoreactivity (plaque and/or diffuse synaptic and/or patchy/perivacuolar types)

B) New variant CJD (vCJD):
- Spongiform encephalopathy with abundant prion protein deposition, in particular multiple fibrillary prion protein plaques surrounded by a halo of spongiform vacuoles ('florid' plaques, 'daisy-like' plaques) and other prion protein plaques, and amorphous pericellular and perivascular prion protein deposits especially prominent in the cerebellar molecular layer

Gerstmann-Sträussler-Scheinker disease (GSS) (in family with dominantly inherited progressive ataxia and/or dementia and one of a variety of PRNP mutations):
- Encephalo(myelo)pathy with multicentric prion protein plaques

Familial fatal insomnia (FFI) (in family with PRNP D178N mutation):
- Thalamic degeneration, variably spongiform change in cerebrum

In the classical form of sporadic prion disease, the most consistent histological feature is spongiform change, a fine vacuole-like appearance in the neuropil (a feltwork of unmyelinated axonal and dendritic neuronal processes within the grey matter of the central nervous system), with vacuoles varying from 20-200 microns in diameter. These vacuoles can appear in any layer of the cerebral cortex and may become confluent, resulting in large vacuoles which substantially distort the cortical cytoarchitecture. Cortical involvement is detectable in most cases, and is usually accompanied by spongiform change in the basal ganglia, thalamus and cerebellar

cortex. Cerebellar involvement is present in most cases as well, although confluent spongiform change is unusual in the cerebellum, and widespread microvacuolar change with smaller vacuoles (20-50 microns in diameter) in the molecular layer is observed. In long-standing cases, the neuronal loss and spongiform change maybe so severe as to result in status spongiosus, where widespread coarse vacuolation results in collapse of the cortical cytoarchitecture, leaving an irregular distorted rim of gliotic tissue containing few remaining neurones. The basal ganglia and thalamus may also exhibit severe neuronal loss with gliosis and atrophy, and in the cerebellum there is often an irregular loss of neurones in the granular cell and Purkinje cell populations. Spongiform change in most brain regions is accompanied by neuronal loss and gliosis involving both astrocytes and microglia, along with deposition of prion protein plaques (Figures 5 a, b and c). Microglial hypertrophy and hyperplasia occur in a widespread distribution, and microglia are implicated in the pathogenesis of prion protein plaques. In the panencephalopathic variant of sCJD, extensive necrotising lesions in the white matter are observed [68].

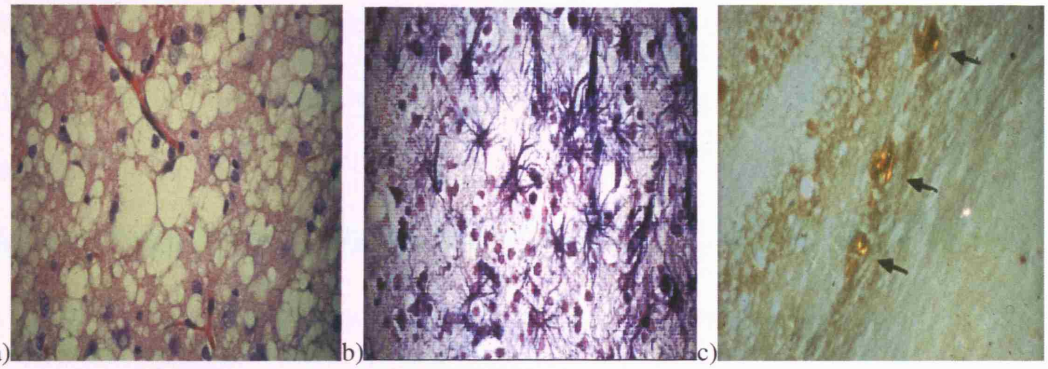


Figure 5: Brain biopsy showing a) spongiform vacuolation b) astrocytosis and c) PrP plaques

The neuropathological findings in inherited prion disease vary according to the PRNP mutation, as described below [69]:

a) E200K: Spongiosis, gliosis, neuronal loss, and PrP plaques, widespread in cerebral and cerebellar cortex

b) D178N: Spongiosis, gliosis, variable neuronal loss and PrP plaques in cerebral cortex, striatum, with thalamus moderately affected

c) Inherited prion disease with insertional mutations: Cases with 4 or fewer extra repeats are indistinguishable from sporadic prion disease, those with 5 to 7 extra repeats may show changes similar to sporadic prion disease, GSS or they cannot be easily classified

d) GSS, most usually described in association with a P102L mutation: Multicentric plaques composed of prion protein are seen, most numerous in the cerebellum, along with neuronal loss, reactive gliosis and gross cerebellar atrophy.

Cerebellar atrophy is also prominent in Kuru, with amyloid plaques seen on histology predominantly in the cerebellum. These plaques are composed of prion protein and are similar to plaques observed in GSS, inherited CJD and sCJD. Variable spongiform change is observed in cerebral cortex, basal ganglia, thalamus and cerebellum.

In iatrogenic prion disease, the characteristic histopathological changes described above are seen. In cases with human pituitary hormone therapy, cerebellar atrophy is marked, with extensive neuronal loss, widespread spongiosis and prion protein amyloid plaque formation.

In variant prion disease, the most striking abnormality is the presence of multiple kuru-type prion protein plaques in the cerebral cortex and cerebellum, surrounded by a halo of spongiform change (Figure 6). Spongiform change is also seen in the basal ganglia and thalamus, with severe thalamic astrocytosis.

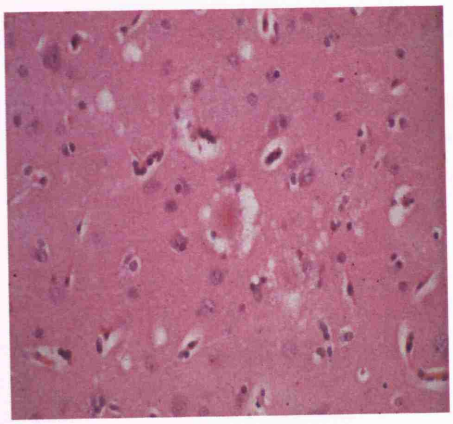
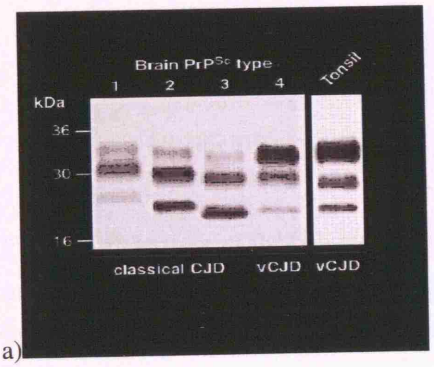
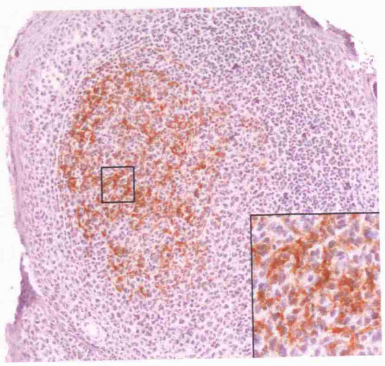


Figure 6: Florid plaques on brain biopsy in vCJD

In addition, diagnosis in vCJD can be established by demonstrating Type 4t PrP^{sc} in a tonsil biopsy specimen, either by prion protein immunohistochemistry (IHC) or immunoblotting (IB) [41, 70](Figures 7 a and b).



a)



b)

Figure 7: Diagnostic PrP^{Sc} analysis of tonsil biopsy tissue by a) immunoblotting and b) immunohistochemical analysis

1.4 PRINCIPLES OF MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging exploits the magnetic properties of hydrogen nuclei, which when exposed to an external magnetic field tend to become aligned in the direction of this field (by convention parallel with the z axis of the relevant coordinate system). The vector sum of the individual nuclear magnetic moments within a region, termed the bulk magnetisation, may be manipulated by applying smaller radiofrequency (RF) pulses, in order to generate detectable signals whose frequency is

linearly dependent upon the main magnetic field strength. The behaviour of the bulk magnetisation following the application of an RF pulse producing a 90° rotation is illustrated in Figure 8 [71].

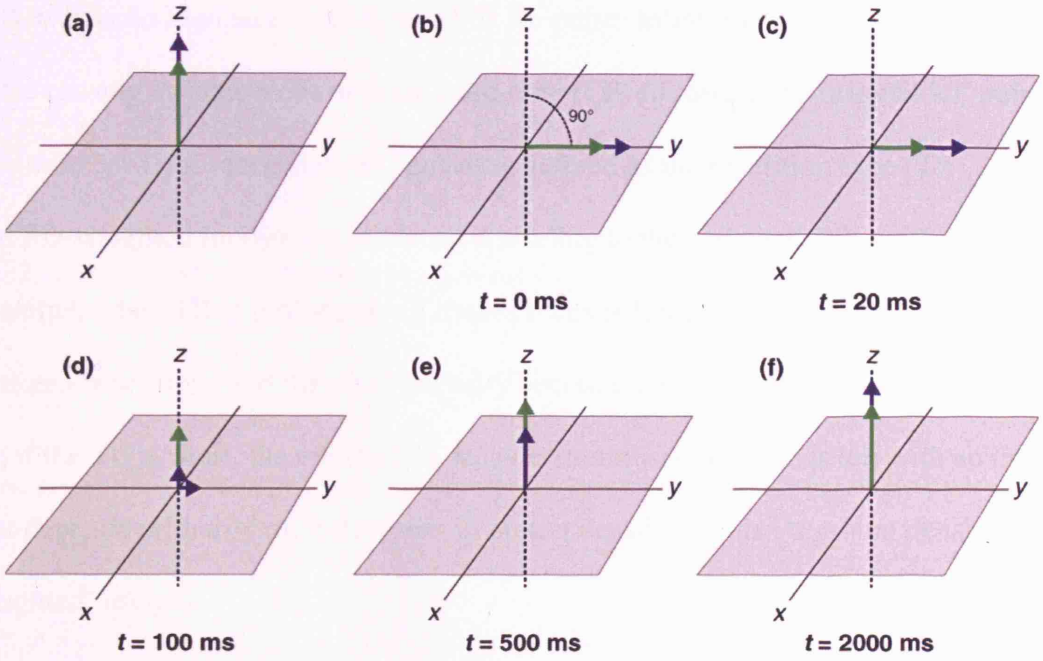


Figure 8: Behaviour of the bulk magnetisation during MRI a) MRI is based on signals originating from nuclear magnetisation induced in the human body when placed in a magnetic field. The figure illustrates the initial magnetisation of hydrogen nuclei (protons) in different tissues (with 2 different exemplary proton densities (PD) here designated in blue and green) aligned along the direction of the main magnetic field (the z axis). b) A 90° radiofrequency (RF) pulse rotates the magnetisation vectors of both tissues into the transverse plane, and their subsequent realignment in the direction of the magnetic field produces the signal used to create the MR image. c-e) Magnetisation returns to its equilibrium alignment along z via 2 exponential processes: spin-lattice relaxation, which determines recovery of the z magnetisation component with time constant (spin-lattice relaxation time) T_1, and spin-spin relaxation which determines decay in the transverse plane with time constant (spin-spin relaxation time) T_2. In this diagram, the green vector has a much shorter T_2 and T_1 than the blue vector so that the green transverse magnetisation vector is seen to decay away faster than the blue, with faster recovery of the green z magnetisation vector. f) Eventually both tissues are relaxed, having returned to their equilibrium states.

Since PD, T_1 and T_2 (each defined in Figure 8) vary with tissue type and pathology, they provide 3 important ways to create image contrast. Images whose contrast

depends on T_1, T_2 and PD are referred to as T_1-weighted (T1WI), T_2-weighted (T2WI) and PD-weighted images respectively. In MRI signals are usually acquired in the form of an "echo" signal which may be generated in two ways:

1. A *spin-echo sequence* starts with a 90° RF pulse, followed by a 180° refocusing RF pulse causing an echo to form at the echo time (TE) following the original 90° pulse. The time between successive 90 ° pulses is defined as the repetition time (TR). T_1, T_2 and PD-weighted images are produced according to the choice of TR and TE (for example, when TR is prolonged, all tissues recover full longitudinal magnetisation between repetitions and the pixel intensity becomes dependent on proton density and T_2; if the TE is short, the effect of T_2 decay is minimised and one is left with an image that depends primarily on differences in proton density, that is, 'a proton density-weighted' image).

Table 4: Choice of TR and TE for spin-echo sequences

TR	**TE**	
	Short (less than 40 ms)	Long (more than 75 ms)
Short (less than 750 ms)	T_1-weighted	Not useful
Long (more than 1500 ms)	PD-weighted	T_2-weighted

2. A *gradient-echo sequence* starts with a smaller RF pulse producing a flip angle (angle of rotation of the average axis of the protons) α less than 90°. TR is again the time between successive α RF pulses and TE is the time from the α RF pulse to the gradient echo which is formed by a reversal of a linear field gradient. Gradient echo sequences use shorter TR and TE, and since the total scan time of an MR sequence depends on TR, they can be acquired much more quickly than spin echo scans.

Appropriate choice of TE, TR and α can produce T_1, T_2, or PD-weighted images (Table 5).

Table 5: Choice of TR, TE and α for gradient-echo sequences

Flip angle α	**TE**	
	Short (less than 15 ms)	Long (more than 30 ms)
Low (less than 40°)	PD-weighted	T_2-weighted
High (more than 50°)	T_1-weighted	Not useful

T_1 and T_2s are different for various tissues in the human body:

1. T_1 for fluids is long (e.g.1500-2000 ms), for water-based tissues is mid-range (e.g. 400-1200 ms), and for fat-based tissues is short (100-150 ms). This distribution of T_1 values can produce excellent anatomical contrast in short-TR T_1-weighted images – fluids are very dark, water-based tissues are mid-grey and fat-based tissues are very bright. T_1-weighted images yield useful anatomical information, as they clearly show the boundaries between different tissues. Pathologies with oedematous characteristics (e.g. in glioma) or an increased capillary density will appear hypointense with respect to the surrounding healthy tissue.

2. Similarly, T_2 for fluids is long (700-1200 ms), for water-based tissues is mid-range (e.g. 40-200 ms), and for fat-based tissues is short (10-100 ms). On T_2-weighted scans, fluids have the highest intensity, and water- and fat-based tissues are mid-grey. T_2-weighted images are commonly considered especially sensitive to pathological tissue changes since any collection of abnormal fluid (e.g. following intracerebral haemorrhage) appears markedly hyperintense against the darker normal tissue.

Although PD-weighted images generally provide less contrast than T1WI or T2WI (as the proton density, or water content, for most tissues is rather similar), they may

still be diagnostically useful: CSF will still be slightly hypo- and fat hyper-intense, e.g. in the cervical spine PD-weighted images show nerve roots well against a dark CSF background.

This phenomenon of nuclear magnetic resonance (NMR) occurs at a characteristic frequency (the 'Larmor frequency') linearly dependent upon the the strength of the specific magnetic field experienced by the nuclei. In order to obtain spatial information, during the MRI procedure the magnetic field strength is made to vary linearly with position, by the superposition of linear magnetic field gradients upon the main magnetic field. The spin- or gradient-echo signal then detected consists of components with a distribution of frequencies, each originating from nuclei at different positions along the magnetic field gradient. This signal may be broken into its component frequencies (by a mathematical technique called a 'Fourier Transform'), the magnitude of the signal at each frequency is proportional to the hydrogen (proton) density at the corresponding location, allowing the spatial concentration of the nuclei to be mapped yielding the final image. Thus, spatial information in MRI is contained in the frequency of the detected signals. Appropriate sequential application of magnetic field gradients in 3 orthogonal directions provides 3-dimensional image information.

1.5 MAGNETIC RESONANCE IMAGING IN HUMAN PRION DISEASES

Magnetic Resonance Imaging (MRI) plays an important role in the diagnosis of human prion disease, as a diagnostic blood test is available only for inherited prion disease and a definitive diagnosis can only be established pathologically with a brain biopsy, or a tonsil biopsy in cases of vCJD.

In vCJD, the characteristic feature on MRI is a positive pulvinar sign, defined by the World Health Organisation (WHO) as bilateral symmetrical pulvinar high signal relative to the signal intensity of other deep grey matter nuclei and cortical grey matter on T2WI, PD- weighted, FLAIR and axial DWI MRI images [72]. The first 2 case reports of pulvinar change were published in 1996 [73, 74], since when the pulvinar sign has been incorporated in the WHO diagnostic criteria for vCJD [37]. This, and the 'hockey stick' sign of high signal in both pulvinar and dorsomedial thalamic nuclei were described in 2000 [40]. Bilateral high signal in the striatum and thalamus has been observed on DWI in vCJD [27]. Occasionally parieto-occipital white matter and asymmetric pulvinar hyperintensity, or cerebral or cerebellar atrophy may be observed [75]. In a study of 36 neuropathologically confirmed vCJD cases compared to 57 controls with suspected CJD, the pulvinar sign had a sensitivity of 78% and specificity of 100% [40]. T2WI and PD images from the most recent set of scans available from each patient were used. In a retrospective study of MRI in 86 neuropathologically confirmed cases of vCJD the most sensitive sequence was FLAIR [75] where the pulvinar sign was present in 100% of the 30 FLAIR scans examined. In a further study of 27 vCJD cases confirmed with tonsil biopsy, compared to 18 tonsil-biopsy negative patients, retrospective blinded analysis of T2WI, DWI, FLAIR and PD-weighted images was performed. A sensitivity of 81% and a specificity of 94% for the pulvinar sign was found [70].

The most common and recognised features of sCJD on MRI are bilateral high signal in the caudate and putamen on T2WI. DWI has been shown to be the most sensitive imaging modality for sCJD [76, 77, 78]. FLAIR has shown pathological hyperintensities in cerebral cortex (called "cortical ribboning") and basal ganglia better than T2WI [79,

[80]. Increased FLAIR signal corresponded to increased DWI signal intensity in basal ganglia and cortex [81, 82, 83].

Imaging appearances in symptomatic patients with inherited prion disease fall broadly into 4 categories: no change [7, 84], cortical atrophy, cerebellar atrophy or decreased T2WI signal in the basal ganglia [84, 85, 86]. Some inherited cases show hyperintensity in the basal ganglia and frontoparietal cortices, similar to sCJD [87]. In 2 series of patients with FFI, MRI did not show any specific abnormality [84] or at most mild to moderate atrophy [86].

In iatrogenic CJD, imaging changes may mimic those of sCJD. The largest and most recent imaging study in an Anglo-French cohort reported bilateral symmetric hyperintensity of the caudate head and putamen in 21/33 patients (64%), DWI changes occurring before T2WI changes [60].

Three cases of possible transmission of vCJD from presymptomatic blood donors have been published [88, 89, 90]. No imaging was reported in a preclinical patient, in 1 symptomatic patient, MRI was normal with no pulvinar sign, and in another symptomatic patient, MRI was initially normal but showed a positive pulvinar sign in the later stages of the disease [90].

1.6 QUANTITATIVE MRI

Quantitative MRI is a scientific approach which allows precise objective measurement of clinically relevant MR parameters, rather than a subjective judgement being made on the basis of unusually bright, dark, small or large objects as in conventional radiology. By quantifying these parameters in individual patients it may be possible to detect changes with disease progression or to determine the effects of therapeutic intervention.

Quantitative MRI includes T_1 and T_2 relaxometry, Volumetric imaging, Magnetic Resonance Spectroscopy (MRS), DWI, and MT [91].

As opposed to T1WI, T_1 relaxometry provides T_1 maps generated from two or more images with different repetition times, flip angles, or inversion times, each producing different T_1-weightings. In patients with MS, the T_1 of normal appearing white matter differs between infratentorial and supratentorial regions [92], suggesting optimal lesion detection may require different T_1 weightings at different levels of the brain.

T_2 maps are generated from two or more T_2-weighted images each with different TEs. Hippocampal sclerosis can be detected in patients with epilepsy by comparing hippocampal T_2 values with values from normal controls.

Generalised or localised changes in tissue volumes in the CNS are sensitive markers of disease progression in several neurological diseases e.g. generalised loss of volume in AD. Although volumetric brain measurements help quantify the extent of disease, they do not give any information about the intrinsic characteristics of the tissue being quantified (i.e. its cellular composition), and magnitude of disease effects can only be measured in combination with other measures of composition.

In addition to simply providing diffusion-weighted images, DWI can also be used to quantify the self-diffusion of water i.e. the random motion of water molecules. Water diffuses easily in all directions in a free environment, such as CSF, but in tissues there are barriers, such as cell walls, which reduce the ability of water to diffuse. The diffusion behaviour of water in the brain is therefore characterised by its *apparent* diffusion coefficient (ADC). The ADC is increased when there is destruction of biological barriers in disease and can be assessed regionally from ADC maps derived from DWI. DWI has been shown to be sensitive in detecting acute ischaemic changes in stroke, and has a high specificity and sensitivity in diagnosing sCJD [93].

Magnetic resonance spectroscopy (MRS) allows non-invasive assessment of specific brain metabolites such as N-acetylaspartate (NAA), reduction of which can serve as a marker of neuronal loss. Decrease in absolute NAA levels, or ratios of NAA levels to those of a reference metabolite, have been seen in variant [94, 95], sporadic [94], iatrogenic [96] and inherited CJD [97].

Magnetisation transfer imaging is described below.

1.7 MAGNETISATION TRANSFER IMAGING

The physical basis of the MT effect is illustrated in Figure 9 [98].

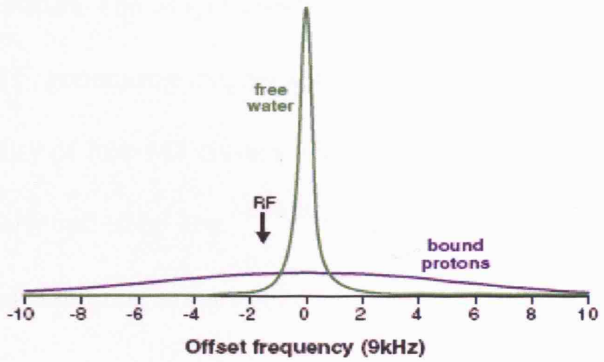


Figure 9: Symbolic representation of the magnetic resonance spectra of free and bound tissue-water

Tissue protons may be considered to reside in two distinct populations: a mobile pool associated with free tissue-water and a bound pool associated with macromolecules [91]. There is constant exchange between the two pools. The signal detected in conventional MRI arises from protons in the mobile pool. If a radiofrequency (RF) pulse is applied which excites the bound pool only (at offsets of 1-2 kHz), and not the mobile pool, this RF pulse has no direct effect on the free-water pool. However, since magnetisation is transferred between the bound and mobile pools, the resulting MT-

weighted image is attenuated and the magnetisation transfer ratio (MTR) can be calculated from this image and a similar one without MT weighting as:

$$MTR=100\ \frac{(M_0 - M_S)}{M_0}\ pu$$

The MRI image intensity is measured in the absence of saturation (M_0) and in the presence of saturation applied to the bound proton pool (M_S) (Figure 9). MTR is the percentage reduction in the signal when saturation is applied, and is expressed in percentage units (pu). Free water and CSF have MTR values close to 0 pu, white matter has a high MTR (30-60 pu), and grey matter has intermediate values [98]. The MTR provides an indication of the quantity of bound protons, and therefore microstructure, in tissues. The MT phenomenon reduces the signal from tissues with large amounts of MT, generating magnetisation transfer contrast (MTC), and thus increases the visibility of low-MT tissues where signal is less reduced; for example, MS lesions. MTR is found to be low [91, 99, 100] in conditions such as MS [3], Alzheimer's disease (AD) [101] and traumatic brain injury [102].

1.8 REGION OF INTEREST (ROI) AND HISTOGRAM ANALYSIS

1.8.1 Overview

This thesis utilizes both region of interest (ROI) analysis to determine MTRs from specific anatomical regions, and histogram techniques to quantify MTR across larger volumes of brain, including whole brain, white matter and grey matter.

The ROI approach allows the study of individual lesions and discrete areas of normal appearing white matter (NAWM) and grey matter (NAGM) [100]. Although anatomically-specific location information is preserved in this analysis, inevitably the number of locations investigated is limited, the choice of location may introduce bias, and significant changes in other unexamined locations may be missed [98]. If multiple

locations are tested, then Bonferroni or other statistical corrections appropriate for multiple comparisons leads to a loss of sensitivity on statistical analysis.

The histogram approach quantifies statistical properties of measurements obtained across extended anatomical regions, or specific tissue types, and may encompass both microscopic and macroscopic lesions in the examined tissue [100]. For each histogram several parameters can be calculated including average MTR, height and location of the histogram's peak and MTR at 25th, 50th and 75th percentile, though the first three parameters are more commonly reported. MTR histograms can be obtained for the whole brain, for specific gross regions of the brain (white matter, grey matter, frontal lobe, cerebellum, and brainstem) and for the cervical cord. MTR histogram analysis is a highly automated technique, avoiding any pre-judgement about which parts of the brain may be affected as is the case in ROI analysis. There is no need to subjectively place ROIs on images. Furthermore, the issue of the reproducibility of ROI positions between subjects and between longitudinally acquired data sets is avoided. Conversely, if the pathology is localised to a specific small anatomical region; for example, in visible lesions, then sensitivity to change will be reduced compared to the ROI approach.

1.8.2 Clinical applications of MTR ROI and histogram analysis

Patients with Alzheimer's disease have demonstrated markedly reduced MTR histogram peak heights for cortical grey matter and temporal lobe grey matter [1]. In this study, a composite MR score based on brain volume and cortical grey matter MTR histogram peak height was correlated with cognitive impairment ($r=0.65$) [1]. Two other studies confirmed this observation, which showed decreased regional mean MTR values for cortical grey matter and temporal lobe grey matter (obtained similarly

to ROI analysis, but using larger areas of interest) in the absence of significant volumetric changes, in patients with mild cognitive impairment (MCI) [103, 104]. Reductions in MTR histogram peak height for whole brain, temporal lobe and frontal lobe have been observed both in patients with AD and MCI. Furthermore, MT-MRI measures from these areas strongly correlated with global cognitive deterioration [2]. In a study which examined MTRs of caudate nuclei, putamen, periventricular white matter and the whole brain in carriers of the Huntington mutation, mild decreases of MTR were observed in the striatum and whole brain [105]. In an ROI study of Parkinson's disease (PD) there was no difference in MTRs of subcortical grey matter and white matter in patients with idiopathic PD and matched controls; whereas patients with PD and dementia had significantly lower MTR in the subcortical white matter, including frontal white matter and genu of the corpus callosum [106]. In another ROI study reduced MTR values were reported in the corticospinal tracts of patients with amyotrophic lateral sclerosis [107].

Reduced whole-brain MTR histogram peak height has been reported in patients with nocturnal frontal lobe epilepsy [108, 109] and focal epilepsy [110] but not in those with idiopathic generalised epilepsy [111].

In patients with normal pressure hydrocephalus ROI MTR abnormalities have been detected in the periventricular normal appearing white matter (NAWM) and in the NAWM of corpus callosum. No abnormalities, however, have been revealed in the thalami [112]. MT-MRI has been used to investigate the nature of brain tumours in vivo. Meningiomas were found to have higher MTR values than other tumours and soft tumours were found to have lower MTR values than hard tumours [113].

Widespread MTR reductions have been reported predominantly in the frontal and temporal cortex but not in the thalami of schizophrenic patients from the earliest stages of disease [114].

Silver et al. reported significant decreases of MTR with increasing age in the corpus callosum and frontal white matter. Studies with histogram-based analyses have reported significant age-related decreases of average MTR and histogram peak height in white matter and grey matter [115-117].

Extensive work in patients with Multiple Sclerosis (MS) has demonstrated a decreased MTR in areas of demyelination [4, 118]. Average lesion MTR has been found to be lower in patients with relapsing remitting MS (RRMS) than in those with clinically isolated syndromes (CIS) suggestive of MS [119, 120]. Low average lesion MTR has also been found in patients with secondary progressive MS (SPMS) and primary progressive MS (PPMS) [121]. Furthermore, a three-year follow up study showed that newly formed lesions from patients with SPMS have a more severe MTR deterioration than those from patients with mildy disabling RRMS [122]. Patients with cognitive impairment have a significantly lower average lesion MTR than do those without; though average lesion MTR explained only 35% of the total variance in neuropsychological test performance [123]. Decreased MTRs have been found, using ROI and histogram analysis, in normal appearing brain tissue (NABT), NAWM [119, 120, 124, 125, 126, 127, 128, 129, 130, 131] and NAGM [126, 132, 133] of MS patients , which correlate with decreased myelin and axonal density [134]. As local MT changes preceded lesion development [135, 136, 137, 138] and normal appearing grey and white matter on conventional MRI showed abnormal MTR values in MS, it was hypothesized that, in CJD, MT will demonstrate changes in normal appearing brain tissue, and may be used

to monitor disease progression; as it has to monitor the effects of interferon beta-1b and intravenous immunoglobulins in MS [139, 140].

1.9 CLINICAL ASSESSMENTS IN DEMENTIA

1.9.1 Dementia Rating Scales

1.9.1.1 Overview

An increasing understanding of the causes and mechanisms of dementia means that more potential therapeutic agents are entering clinical trial phases than ever before. Clinical trials require careful design in order to sensitively detect pathological change and the efficacy of new treatments. Where possible, randomised controlled trials should be used (double blinded and placebo controlled) with entry allowed to those patients who fulfil established diagnostic criteria. In addition, the aims of the trial should be clear; for instance, either halting decline or showing improvement. In dementia the choice of rating scale (the outcome measure) is critical. A common problem is that treatments may only be useful in a subset of the patients with a disease. Understanding the normal progression of decline with a set of clinical scales is thus paramount, so that any change from the background non-treated disease population can be detected. It is important to have the ability to assess a range of treatment responses, both in groups and individuals. The multifaceted nature of dementia means that many functional areas need to be assessed such as physical and social ability, behaviour, emotional response and relationships, overall function, as well as cognition.

Many dementias have their own disease specific scales, tailored to the disease, but none are specific for prion disease. A number of well established and widely used rating scales allow comparison between the dementias as well as between different

treatments in the same disease. Some of these were thus employed in the present study for the first time to assess patients with prion disease in a therapeutic trial in order to assess the feasibility of these scales for long-term use in future trials.

Outcome measures can be organised into five groups:

(1) Cognitive tests

(2) Clinicians' global assessments of severity

(3) Clinicians' global assessments of change

(4) Behavioural ratings

(5) Functional assessments

(6) Comprehensive rating scales

1.9.1.2 Cognitive tests

Cognitive tests are often the primary measure of efficacy in clinical trials. Measures used include the Alzheimer's Disease Assessment Scale (ADAS) [141], the Syndrom Kurztest (SKT) [142, 143], the Information-Concentration-Memory subtests (ICM) from the Blessed-Roth Dementia Scale [144], the Mattis Dementia Rating Scale (DRS) [145], and the Mini Mental State Examination (MMSE) [146].

Most recent clinical trials for AD have used the cognitive subscale of the ADAS (ADAS-COG) to measure cognitive change. This was designed as a battery of tests to assess impairments seen in AD and takes approximately an hour to perform. In AD the ADAS-COG has revealed significant differences in randomised controlled trials with cholinesterase inhibitors and other drugs.

The Syndrom Kurztest (SKT) assesses attention and memory using 9 performance

subtests, each limited to 1 minute, and has been used predominanatly in clinical trials in German-speaking countries. The SKT has been used as a cognitive outcome measure in clinical trials of various drugs aimed at enhancing cognition.

The Mini Mental State Examination (MMSE) was developed as a cognitive screening test, but has proven to be useful in staging and is sensitive to change. It takes around 10 minutes to perform.

Other tests often used in dementia include the Information-Concentration-Memory (ICM) Test which uses components of the Blessed-Roth Dementia Scale and the Cambridge structured interview to assess orientation, long-term memory, recall, concentration and performance. The Mattis Dementia Rating Scale scores attention, perseveration, praxis, abstraction, verbal and non-verbal recent memory.

The Severe Impairment Battery (SIB) [147] was developed for severely impaired patients and uses single words or one-step commands combined with gestures. Its use is similar to the Severely Affected Protocol used in this thesis; i.e., those with MMSE less than 10 unable to perform the ADAS-COG or MMSE.

Problems may be encountered when scores 'floor out', thus scales aimed at the later stages of disease have been developed. The Level of Cognitive Functioning Scale (LCFS) [148] is one of the early scales used to assess cognitive functioning in post-coma patients (Hagen (1972)). It was developed for use in the planning of treatment, tracking of recovery and classifying of outcome levels. Eight levels are used to classify patient response from I-No response to VIII-Purposeful-appropriate. It is also useful in tracking the opposite situation of decline.

1.9.1.3 Clinicians' global assessments of severity

Assessing severity helps the clinician to give prognostic information and plan optimal

care. It can also be used to stage entry into clinical trials. These tests often encompass a wider range of symptoms than cognitive tests and are thus a better way of tracking disease progression.

Examples of these tests include the Clinician's Global Impressions-Severity of Illness scale (CGIS) [141], a non specific 7-point scale ranging from 1(normal) to 4 (moderately ill) to 7 (among the most extremely ill). The Clinical Dementia Rating Scale (CDR) [149] is a 6 point scale determined by structured interview with patient and carer. It either stages dementia, including cognitive, functional and social assessment or measures change by summing scores. The Global Deterioration Scale (GDS) [150] is a 7 point staging of dementia (1=normal, 3=late confusion, 5=middle dementia, 7=late dementia) that does not require an interview.

1.9.1.4 Clinicians' global assessments of change

This category of clinical assessments characterises change from a specified baseline. The rationale is that a clinician should be able to see the clinical effect of a treatment after a short interview. These measures are fairly unstructured which limits their sensitivity. The most commonly used measure is the Clinician's Global Impression of Change (CGIC) [151] which has been used in many early clinical trials of anti-dementia drugs. An impression of change is scored by the clinician from 1= very much improved to 7= very much worse. Both the CIBIC (Clinicians' Interview-Based Impression of Change) [151], which uses a similar 7 point scale to the CGIC, and the CIBIC+ which additionally interviews the caregiver as well as the patient are also commonly used, but are more in depth interviews than the CGIC.

1.9.1.5 Behavioural ratings

Behavioural ratings are not often used in clinical trials as patients are often selected on the basis of those who do not have significant behavioural symptoms such as agitation, depression, delusions and hallucinations. The most commonly used scale is the ADAS non-cognitive behaviour subscale [141] which assesses tearfulness, depression, concentration, uncooperativeness, delusions, hallucinations, pacing, motor activity, tremors and appetite on a 6-point (0 to 5) severity scale. The Behavioural Pathology in AD scale (BEHAVE-AD) [150] assesses 25 well-defined behaviours in 7 areas, rated as mild, moderate, or severe. The Brief Psychiatric Rating Scale (BPRS) [152] uses an interview by an experienced clinician; 18 items are rated on a seven-point severity scale. The Neuropsychiatric Inventory (NPI) [153] assesses thirteen behaviours on the basis of frequency and severity.

1.9.1.6 Functional assessments

Activities of daily life are an important and practical assessment of a patient's ability to maintain independent living. Assessments are usually performed with both carer and patient present. The Rankin [154] and Barthel [155] scores are most often used currently in clinical trials, the former due to its brevity and the latter due to its good assessment of longitudinal function. In the past, the Physical Self-Maintenance Scale (PSMS) and the Instrumental Activities of Daily Living (IADL) [156] were used with newer tests such as the Progressive Deterioration Scale [157] and the Interview for Deterioration in Daily living activities in Dementia (IDDD) [158] adding to the areas tested and the number of rating points, structuring the interview.

1.9.1.7 Comprehensive rating scales

These scales assess patients on a range of cognitive, behavioural, motor, and daily

activities. Examples include the Gottfries–Brane–Steen (GBS) [159] and Sandoz Clinical Assessment Geriatric (SCAG) scales [160]. The GBS is a 26-item, 7-point scale assessing motor performance, intellectual impairment, emotional impairment and other symptoms. The SCAG includes 18 cardinal signs and symptoms of dementia rated by the clinician.

The Disability Rating Scale (DRS) [161] was developed for patients with moderate and severe traumatic brain injury to accurately measure general functional changes in an inpatient setting. The DRS can track an individual from coma (extreme vegetative state=29) to community living (without disability=0). Measurement areas include eye opening, communication, motor response feeding, toileting, grooming and employability. Although the DRS is insensitive at the low end of the scale and cannot detect subtle changes, it is useful in measuring decline once symptoms are established.

The heterogeneity of disease in dementia means that many scales have been devised in an attempt to capture the stages of decline that are clinically significant and that measure change in treatment trials. Much can be learnt from the different scales applied to dementia and head injury as there is significant crossover. Due to current paucity of such disease-specific scales it is vital that those most appropriate for prion disease be determined to provide outcome measures in future clinical trials.

1.9.2 Clinical Video in Neurology

Video has an increasing role to play in neurology. Imaging of movement disorders has proved useful in the diagnosis and analysis of movement patterns and to track symptom progression and treatment. EEG video monitoring is used in diagnosis of epilepsy and sleep studies use video to assess movement during sleep. Clinical

teaching has been augmented with examples of clinical cases and signs demonstrated on video, including online video publications which allow immediate visualisation of clinical signs in patients. Video conferencing is becoming more commonplace, allowing multiple audiences to see clinical demonstrations.

Motor function can also be assessed with relative ease using video examinations. In this study various measures of motor function were videoed, including walking, heel-toe walking, rapidly alternating movements, copying gestures and sequential finger tapping.

Use of video in gait analysis in dementias with motor signs such as Huntington's disease has been previously reported [162], as has the use of video to assess the effect of clozapine within a trial setting (double-blind randomised comparative study) in improving the voluntary and involuntary motor signs in Huntington's disease [163]. One of the characteristic motor signs in prion disease is myoclonus. Video has been used to characterise myoclonus in other dementias, such as Rett syndrome [164]. Articles have been published [165] reporting the satisfactory use of hand held video cameras for imaging the face and anterior eye, a technique similar to that used in the current study.

The Mini Mental State Examination (MMSE) [146] is one of the non-videoed tests employed in this thesis. Various publications have discussed the use of 'remote' scoring of the MMSE (e.g. videoconferencing, fax or telephone consultations) versus face to face scoring. Although videoconferencing is different from simple video, as images are viewed in real time, the same principles should apply.

Ball et al [166] assessed the reliability of scoring written items of the MMSE (sentence and pentagrams). Face to face and fax were the most reliable, videoconferencing scored sentences reliably, but not pentagrams. They, therefore, advised caution in accepting video results. In an earlier study Ball [167] found that written material viewed

via videoconferencing was difficult to score, but that rescoring the written material 'in person' did not move any subjects into or out of the range suggesting cognitive impairment.

Montani et al [168] reported the performance of 15 hospital inpatients, all over the age of 75 with no known psychiatric history, on MMSE. They found a small but significantly lower performance on MMSE when using a videoconferencing system as compared with face to face examination. Some patients also felt inhibited by the presence of the equipment and sound quality was sometimes poor. In a study [167] of MMSE in a young adult psychiatric population some patients showed higher attention levels in the video consultation than when face to face but there was good correlation between the scores. In one type of cognitive assessment (CAMDEX) [169] technical video problems meant that written test responses could only be scored in person and not from video. Other tests of cognitive function (CAMCOG) [170] have been used reliably over a videoconferencing system without major modification.

Within the PRION-1 trial a BPRS (Brief Psychiatric Rating Scale) [152] assessment was videoed with the patient to allow later scoring by an independent psychiatrist. The experience of videotaping the BPRS has been described in 2 papers [171, 170].

Other psychiatric ratings using video have been published. In Teri et al [172] caregivers assessed depression in dementia patients using video. They found that caregivers were able to correctly identify depression in patients and that the caregiver's mood did not affect this assessment. Fuchs et al [173] describe a double-blind, placebo-controlled trial of the antidepressant maprotiline in patients with dementia and depression where a videoed rating of global impression was used as well as the Mini-Mental State Examination and Geriatric Depression Scale [174]. Here video rating was the main

parameter of the study and interrater reliability was high. A review of the use of digital video in psychiatry is published in Falzone et al [175].

Memory is assessed using video in patients with dementia of the Alzheimer type in Rusted et al [176]. In this longitudinal study performance in tea-making was assessed in the home setting, and in unfamiliar surroundings, over a 6 year period, and though large differences were observed in the rate of decline of patients, the major change was an increase in omission of actions habitually completed as a part of tea-making routine.

2. METHODOLOGY

2.1 SUBJECTS

Subjects studied for this thesis were those patients recruited from the National Prion Clinic with inherited, sporadic and variant forms of human prion disease who took part in the MRC PRION-1 trial and who underwent MT imaging. Ethical approval for the study was obtained from the Eastern Multi Regional Ethics Committee (MREC). Informed consent was obtained at enrolment from either the patient or patient's next of kin and consent for clinical assessments and MRI was reconfirmed at each examination time point. The first clinical assessment included in the work reported in this thesis was that performed at the same time as the first MRI scan. Statistical comparisons of clinical and MRI assessments obtained at baseline were performed, as well as longitudinal analyses.

2.2 MRI EXAMINATION

Section 2.2 describes the protocols for the acquisition of the MT, T1-weighted, DWI and FLAIR image data sets.

2.2.1 MRI procedure

The process of performing an MRI examination was defined in a standard operating procedure (SOP) (Appendix A). Each patient was given a patient information sheet on MRI (Appendix B). Patients were scanned at the National Hospital for Neurology and Neurosurgery, London, WC1N 3BG, and MRIs were performed at 0, 1, 2, 4 and 6 months from enrolment in the study, and thereafter three monthly (see Figure 10-PRI0N-1 trial clinical assessment and MRI schedule for follow up).

													repeat 3 monthly		
Evaluation (month)	**Screen -ing***	**Entry (0)***	**1**	**2***	**3**	**4**	**5**	**6***	**7.5**	**9***	**10.5**	**12***	*13.5*	*15**	**Ouina-crine discont-inuation**
Local assessment[a]					X		X		X		X		X		
Informed Consent	X	(X)													
Screen for eligibility /ineligibility criteria	X														
Complete general medical history and physical exam	X														
Brief history and physical exam		X	X	X		X		X		X		X		X	X
Full blood count[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Biochemistry[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical examination (digital visual recording)		X	X	X		X		X		X		X		X	X
CIBIC-plus, BPRS		X	X	X		X		X		X		X		X	X
Other neurological assessments		X	X	X		X		X		X		X		X	X
Specimen storage[d]		X	X	X		X		X		X		X		X	X
Plasma drug levels[e] (if patient taking quinacrine)			X	X		X		X		X		X		X	X
OPTIONAL INVESTIGATIONS[f]															
CSF exam[g]		X	X	X				X				X		X	
Tonsil biopsy (vCJD only)	(X[h])			X											
MRI		X	X	X		X		X		X		X		X	
EEG		X	X	X		X		X		X		X		X	

Note: confirm consent at trial entry
[a] Visit for patients with inherited human prion disease preferring not to receive quinacrine.
[b] full blood count and biochemistry performed locally and a copy of results sent to the National Prion Clinic
[c] haemoglobin, white cells, neutrophils, lymphocytes and platelets
[c] sodium, potassium, urea, creatinine, AST or ALT, alkaline phosphatase, bilirubin, GGT, amylase
[d] At least 30ml, and wherever possible 50ml, EDTA blood for plasma storage and 24-hour urine sample
[e] 10ml blood for plasma store for quinacrine assay
[f] consent for these optional research investigations is not required for entry into PRION-1, and will be re-confirmed at each timepoint
[g] cell count, protein, glucose,14-3-3 immunostaining, NSE, S-100b.
[h] if not previously performed and not contra-indicated.

Figure 10: PRION-1 trial clinical assessment and MRI schedule for follow-up

To minimise motion artefact in patients with involuntary movements, especially in those in relatively late stages of the disease, the following measures were adopted:

a) MT imaging, with an acquisition time of 12 minutes, was initially carried out towards the end of the PRION-1 MRI protocol, which required 35 to 40 minutes for completion, making it difficult for cognitively impaired patients or those with myoclonus to remember instructions and to lie still. Subsequently this was changed such that MT imaging was carried out in the earlier half of the protocol in an attempt to minimise motion artefacts.

b) To minimize head motion a vacuum headcap consisting of a two-layered hood containing many soft polystyrene beads was used. This hood was placed around the

patient's head and the beads distributed to form an even layer around the head. The air within the hood was evacuated with a single hand pump. The beads collapsed on themselves forming a rigid mould of the patient's head. Although this did not completely stop the patient from moving their head, there was only one head position in which they were really comfortable, towards which they tended to return. This could not be used for some patients who felt claustrophobic.

c) Sedation with an oral sedative was sometimes helpful in patients who were unable to tolerate a vacuum headcap. Patients were monitored throughout the scan, but in some cases oral sedation was associated with rebound agitation, leading to motion and compromising the quality of the scan.

d) When all the above measures failed in some patients, general anaesthesia (GA) was given. A Standard Operating Procedure was used (see Appendix C) and consent for MRI and GA was reconfirmed at each scan with either the patient or their next of kin (see MRI consent form in Appendix D). MRI under GA was carried out at the discretion of the treating clinician, with priority given to main assessments at months 0, 2, 6 and 12.

2.2.2 MRI acquisition and pre-processing

Scans were performed by radiographers at the National Hospital for Neurology and Neurosurgery Neuroradiology department. The full scanning protocol for the trial included MT, DWI/ADC, T1 Volumetric imaging (from T1-weighted coronal images), FLAIR, and MRS, the latter not being considered further in this thesis.

All MRI scans were acquired on a 1.5-Tesla Signa Echospeed Horizon system (GE Healthcare, Milwaukee, WI, USA) using the standard transmit/receive head coil.

Magnetisation transfer imaging was performed using an interleaved 2D-gradient-echo

sequence [177]. Thirty slices of 5mm thickness and 1.5mm separation were acquired. Matrix size was 256x192, field of view (FOV) 24x18cm, number of excitations (NEX) 0.75 and flip angle 70° with total acquisition time 12 minutes. The presaturation pulse was a Gaussian pulse with a duration of 12.8 milliseconds and a peak amplitude of 23.2 μT giving a nominal bandwidth of 125 Hertz, applied 2 kHz off water resonance. The energy deposited by this pulse provided measurable differences between saturated and unsaturated images and ensured a good contrast to noise ratio in the MT image. To ensure exact co registration of the pixels on saturated and unsaturated images, scans with and without presaturation were interleaved for each TR period [178]. In addition, diffusion-weighted (30 contiguous 5 mm axial slices, TE 100 ms, TR 10000 ms, 1 NEX, 96×128 matrix, FOV 26×26 cm, diffusion-weighting factor (b) =1000sec/mm^2, flip angle 90°, acquisition time 1 min), FLAIR (24 contiguous 5 mm axial slices, TE 161, inversion time (TI)=2473 ms, TR 9897 ms, 1 NEX, 256×192 matrix, FOV 24×24 cm, flip angle 90°, acquisition time 4 minutes) and 3D inversion-recovery prepared T1-weighted image (124 contiguous 1.5mm coronal slices, TE 6 ms, TR 14 ms, TI 600ms, 1 NEX, 256x128 matrix, FOV 24×18 cm, flip angle 15°, acquisition time10 minutes) images were also acquired.

After each scan the radiographer completed a PRION-1 Trial MRI Proforma (Appendix E). Prior to off-line analysis, the MT and T1-weighted coronal data sets were anonymised by a physicist and transferred from the hospital Picture Archiving and Communication Systems (PACS) to a GE Advantage Windows workstation or Sun Blade 1500 workstation (Sun Microsystems, Mountain View, California) via file transfer protocol (FTP) for analysis. FLAIR and DWI sequences were retained on PACS for further radiological assessment.

2.3 MTR ANALYSIS

Section 2.3 describes the two approaches which were adopted to determine regional and global MTR values, and their independent quality control processes:

1. Region of Interest (ROI) analysis
2. Whole brain, grey matter and white matter MTR histogram analysis

2.3.1 ROI analysis

Magnetisation transfer ratio (MTR) maps were generated from the raw MT images produced with the presaturation pulse on, and those with the pulse off (Figure 11).

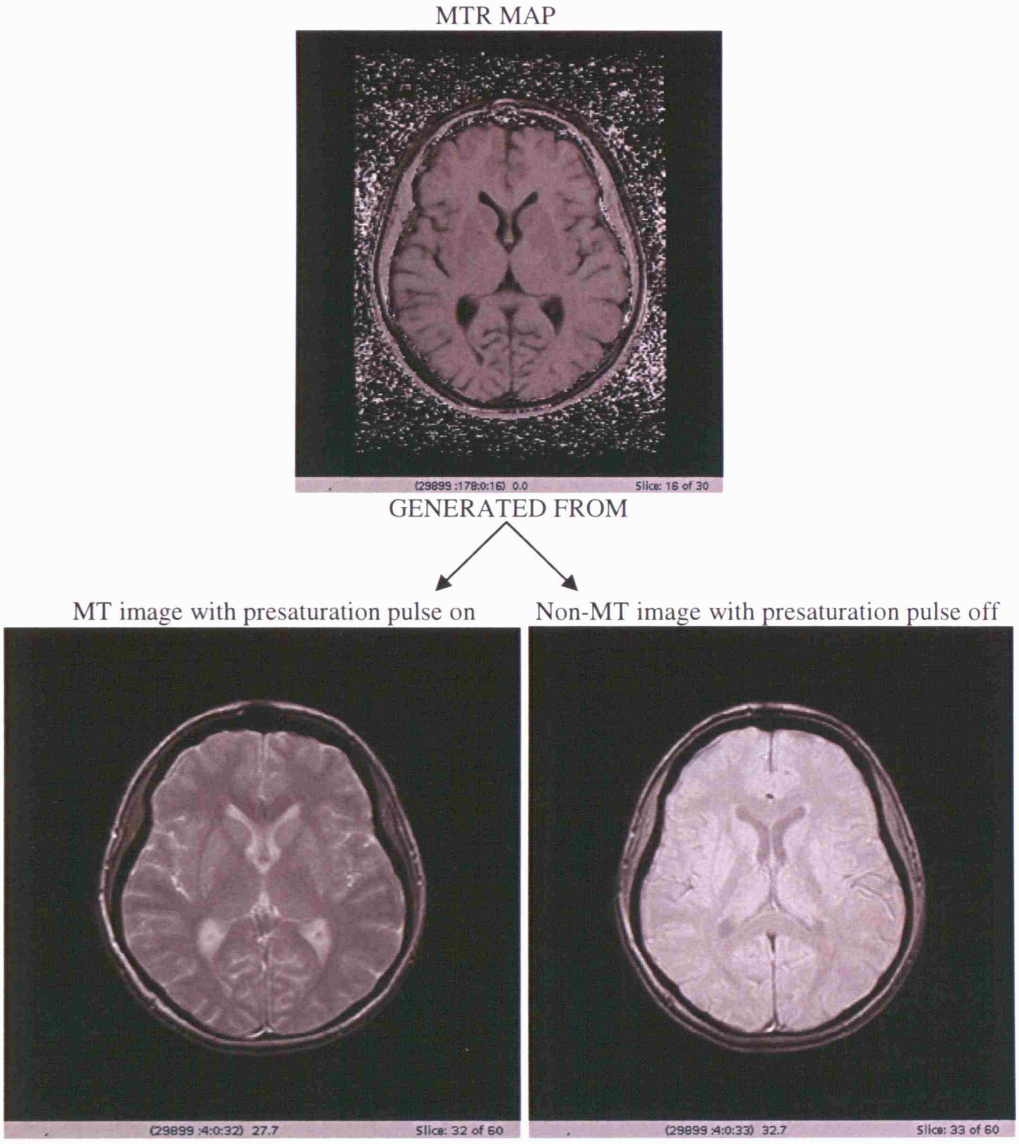


Figure 11: Generation of an MTR map

Using GE Functool software MTR was calculated for each pixel by the formula MTR = ([M_0 – Ms]/ M_0) ×100 percent units (pu) where Ms, and M_0 represent signal intensities for each pixel with and without presaturation, respectively. Prior to the calculation, all pixel values were multiplied by 1000 to minimize integer rounding errors. All MTR values are reported herein as percentage units (pu) scaled to lie between 1 and 100. MT images were displayed on a Sun workstation and Region of Interest (ROI) analysis was carried out using DispImage display and analysis software [179], in a blinded randomised manner, with 33 anatomically distinct ROIs (Figure 12) manually defined by a single researcher (myself). After training on the Sun workstation, intrarater and interrater variability was calculated. The mean intrarater difference was 0.41%, SD 0.72%, with the maximum likely difference between repeat readings for intrarater agreement being 1.42%. The mean interrater difference was 0.49%, SD 1.01%, with the maximum likely difference between repeat readings for interater agreement being 1.98%. For the purpose of calculating the interrater difference, 5 scans were analysed by myself and another researcher. All of the remaining analysis was performed by myself alone.

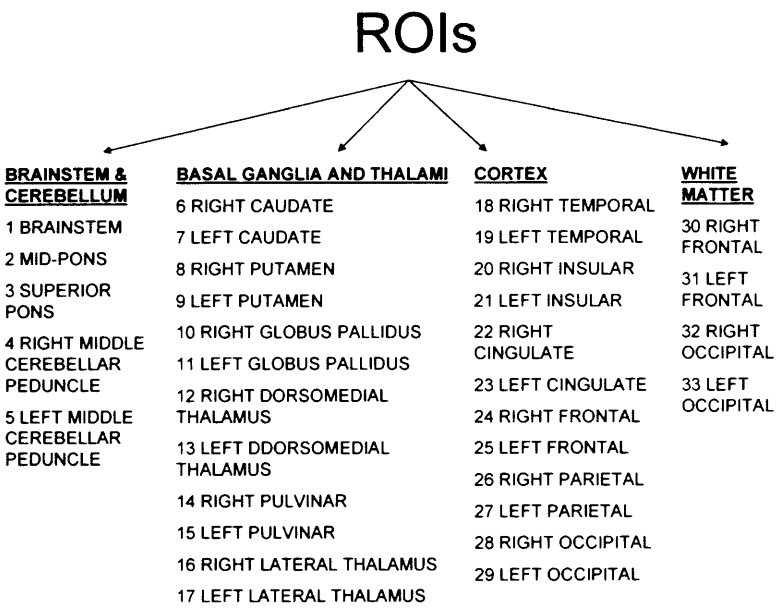
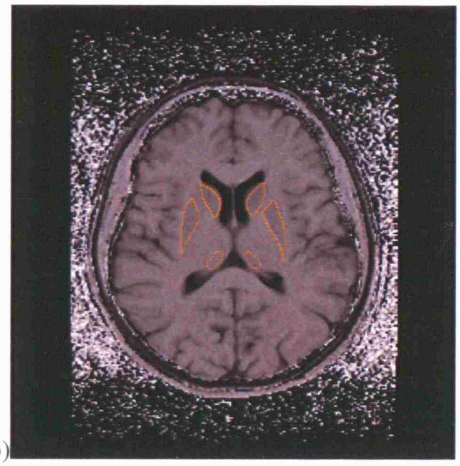
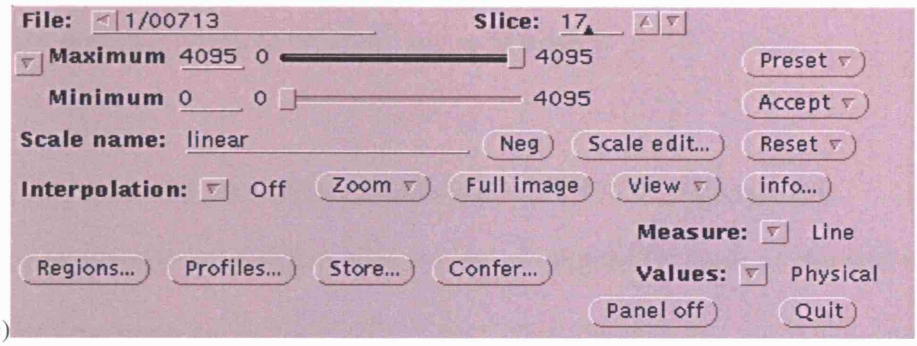


Figure 12: Regions of interest (ROIs)

Regions of interest were outlined on the MT images and then transferred to the inherently co-registered MTR maps for ROI mean MTRs to be calculated (Figure 13).

a) 

b) 

c) 

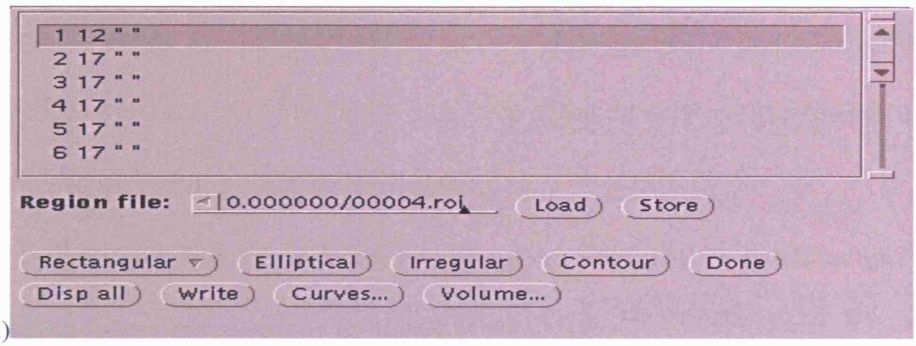
d) 

Figure 13: Bilateral caudate nuclei, putamina and pulvinar ROIs drawn on a) axial MT image and b) axial MTR map for calculation of mean MTR for each ROI, using c, d) DispImage software

Originally 33 different ROIs were considered, including both the grey and white matter. Preliminary investigations showed that it was difficult to accurately define the cortical ROIs without partial-volume contamination from adjacent white matter and

cerebrospinal fluid (CSF). These cortical ROIs were, therefore, excluded from further analysis. Since human prion disease predominantly affects the grey matter, white matter ROIs were also excluded and 6 grey matter areas included in the final analysis; both caudate nuclei, the putamina and bilateral pulvinar regions. The ranges in area of the various ROIs and their standard deviations were:

a) Right caudate nucleus (size=40-121 mm^2, mean SD=2.14)

b) Left caudate nucleus (size=41-146 mm^2, mean SD=2.07)

c) Right putamen (size=124-400 mm^2, mean SD=2.16)

d) Left putamen (size=96-347 mm^2, mean SD=2.03)

e) Right pulvinar (size= 44-65 mm^2, mean SD=1.75)

f) Left pulvinar (size=44-65 mm^2, mean SD=1.85)

2.3.2 Whole brain, grey matter and white matter MTR histogram analysis

In whole-brain histogram analysis a histogram of pixel MTR values is formed from the whole of the brain parenchyma. Thus, both focal damage and more widespread diffuse tissue damage are reflected in changes to the shape of the histogram, with a general shift toward lower MTR values expected if the density of macromolecules is reduced due to underlying disease processes.

The generation of a whole-brain MTR histogram requires that the brain parenchyma be segmented from cerebrospinal fluid and other tissues [180, 126, 125, 132, 117, 122]. This segmentation procedure was accomplished automatically by a single researcher (myself) in a blinded, randomised manner using validated third-party software for image manipulation and format conversion (Jim version 4.0 (www.xinapse.com/software.html)) and tissue segmentation (FSL v3.2 (www.fmrib.ox.ac.uk/fsl)) in order to generate whole brain, grey matter and white

matter histograms. To avoid bias the segmentation was performed using the 3D inversion-recovery prepared T1-weighted data, which also demonstrated superior grey-white matter contrast rather than the MT images. Image registration of the MT data was achieved using the non-MT images rather than the actual MTR maps, since the former demonstrated significantly higher grey-white matter contrast improving registration accuracy. The individual steps in the histogram analysis procedure are summarised as follows:

a) Axial MTR maps generated from raw axial MT- and non-MT-weighted images.

b) 3D Coronal inversion-recovery prepared T1-weighted images resliced to provide axial images with similar orientation to the MT images, the IR-SPGR images providing better grey-white matter contrast for effective segmentation of CSF, whole brain, white and grey matter.

c) Extracerebral tissue segmented and removed from the MTR maps, non-MT, PD-weighted images and axial inversion-recovery prepared T1-weighted images.

d) Axial T1-weighted images segmented into whole brain and CSF (for whole brain histograms), or into white matter, grey matter and CSF (for grey and white matter histograms).

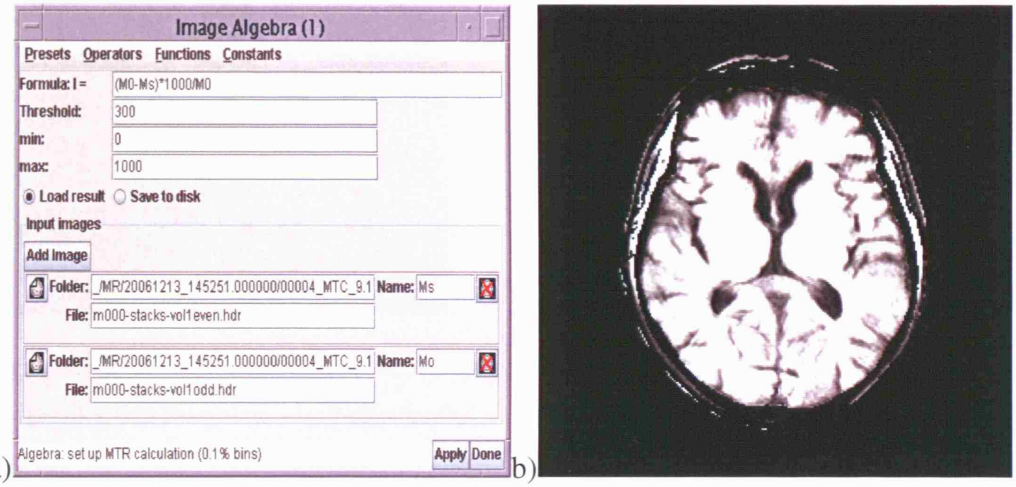
e) Non-MT PD-weighted images co-registered to axial inversion-recovery prepared T1-weighted images, such that the parenchyma, grey matter and white matter segment masks generated in d) could be applied to the MTR images.

f) Geometric transformation obtained in e) applied to the MTR maps, to obtain masked MTR maps containing parenchyma, grey matter and white matter segments only.

g) Normalized MTR histograms were generated for each tissue class obtained in f) and histogram measures calculated.

The details of these steps are as follows:

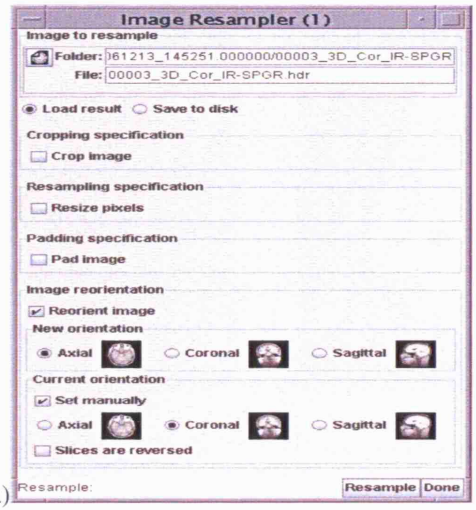
a) Jim 4.0 was used to generate axial MTR maps from axial MT images and axial non-MT, PD-weighted images using the formula given above (Figure 14).



a) b)

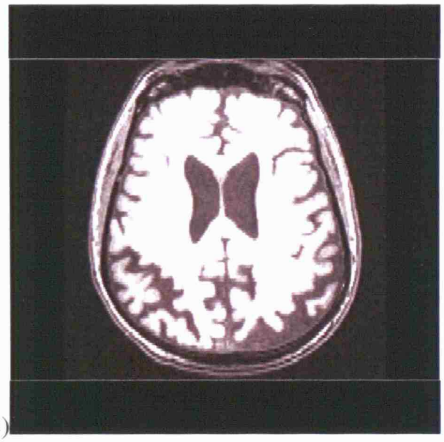
Figure 14: Generation of axial MTR map a) using image algebra b) in Jim version 4.0

b) Jim 4.0 was also used to reslice the coronal inversion-recovery prepared T1-weighted images acquired in the same imaging session as the MT data to provide axially oriented images (Figure 15) with an orientation similar to the MT images. The axial T1-weighted images provided a better grey-white matter contrast compared to axial MT images, and were therefore used to provide optimal segmentation of CSF, whole brain, white and grey matter.



a)

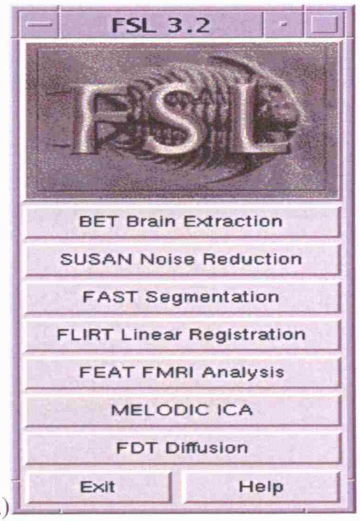
b)



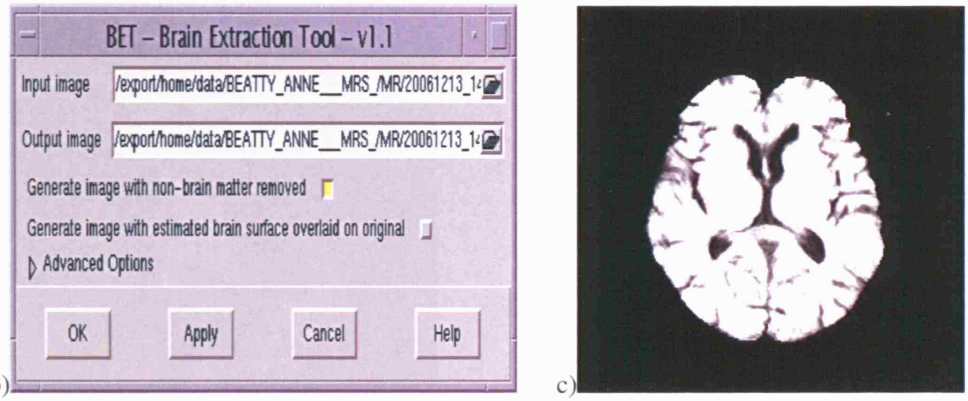
c)

Figure 15: a) Image Resampler used to convert b) coronal inversion-recovery prepared T1-weighted image to c) axial T1-weighted image

c) Then in FSL, Brain Extraction Tool (BET) [181] was used to exclude extracerebral tissue from the MTR maps, non-MT, PD-weighted images and the axial T1-weighted images (Figure 16).



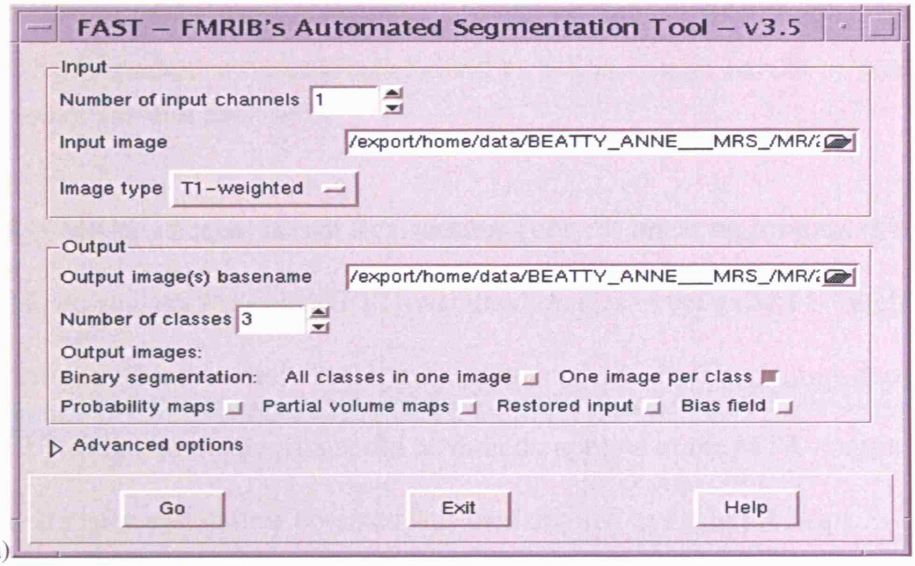
a)



b) c)

Figure 16: FSL a) BET b) used to remove extra-cerebral tissue in an axial MTR map c)

d) FAST (FMRIB's (Oxford centre for Functional Magnetic Resonance Imaging of the Brain) Automated Segmentation Tool) [182] segmentation in FSL was used to segment the axial T1-weighted images into either whole-brain and CSF (for whole-brain histograms) or into white matter, grey matter and CSF (for grey and white matter histograms) (Figure 17).



a)

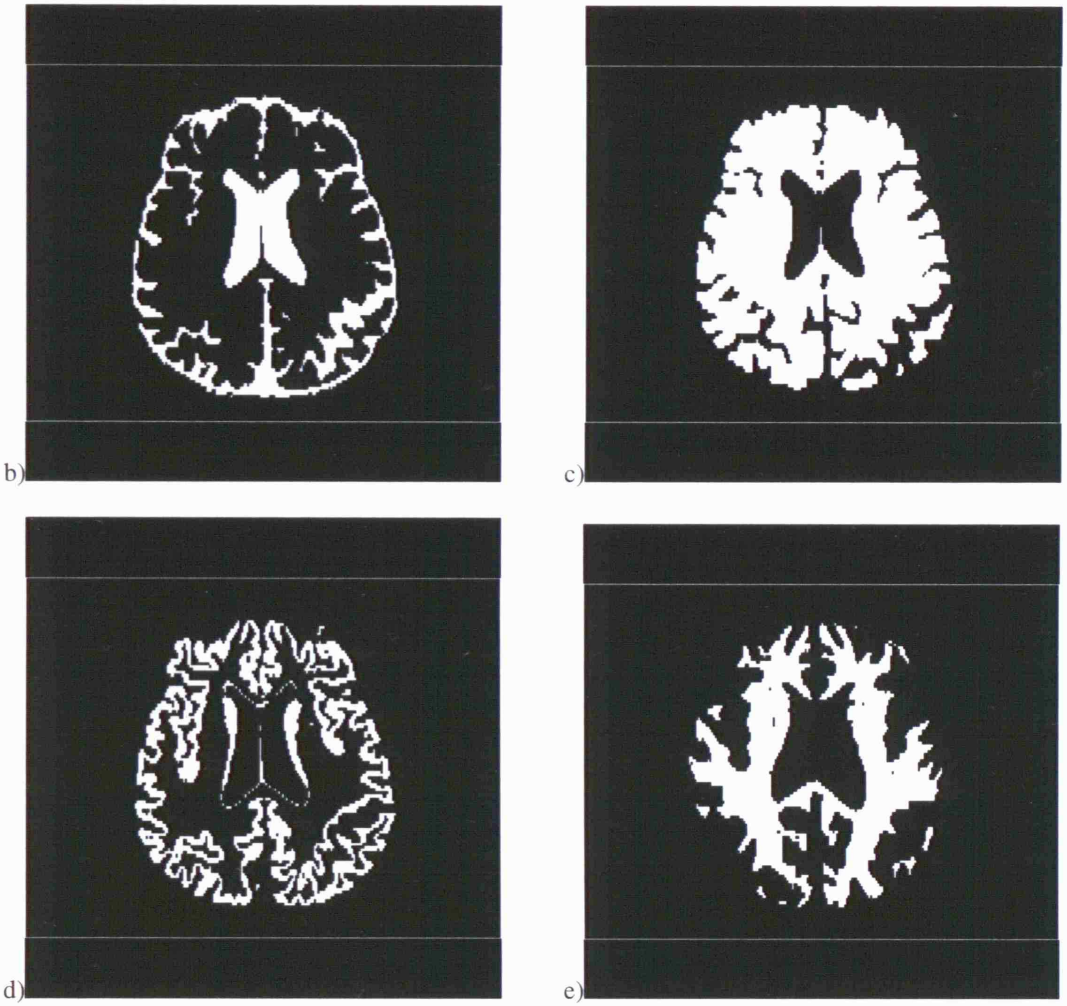


Figure 17: FAST segmentation a) used to segment axial T1-weighted images into CSF b), whole brain c), grey matter d) and white matter e)

e) FLIRT (FMRIB's Linear Image Registration Tool) [183] linear registration in FSL was used to co-register the non-MT PD-weighted images to the axial T1-weighted data set, in order that the whole-brain, grey matter and white matter masks derived from the T1-weighted image data could be directly applied to the MTR images. The geometric transformation thus obtained was then applied to the MTR maps.

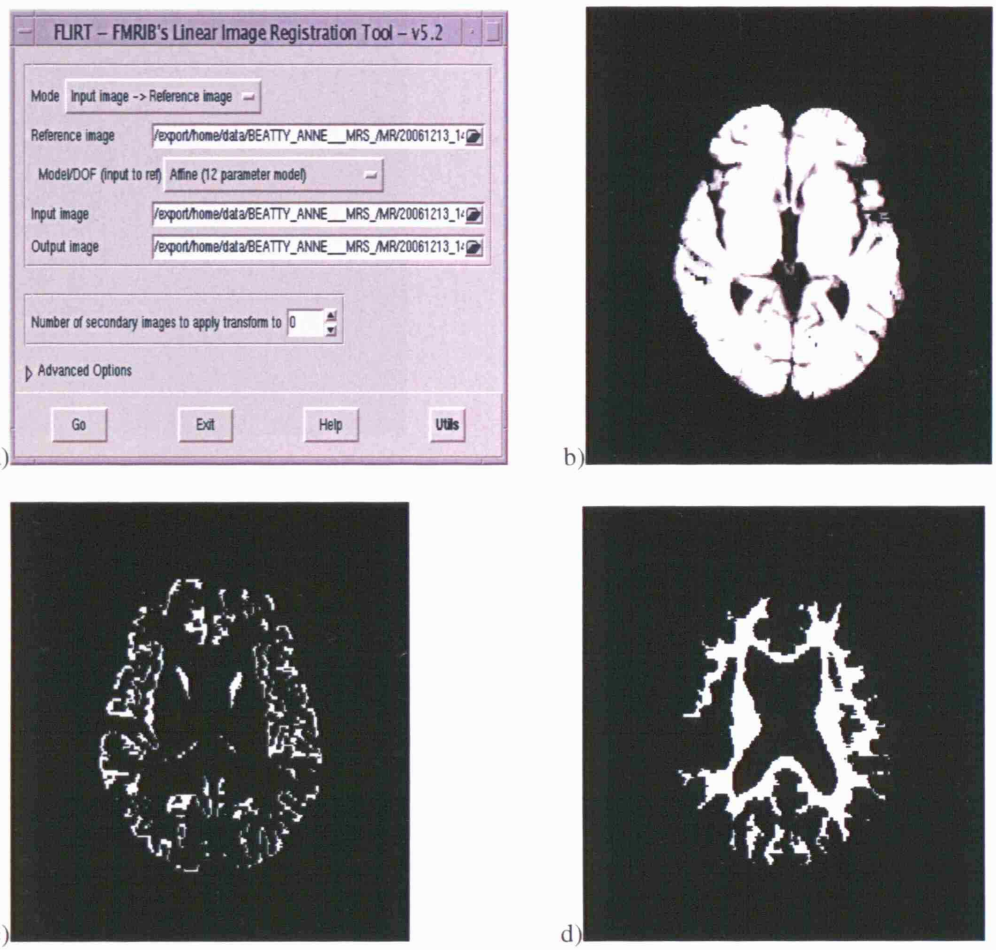
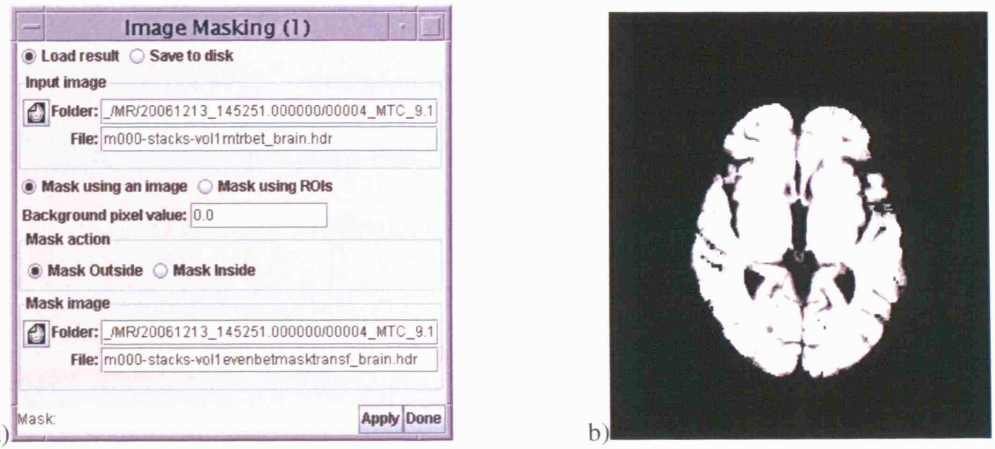


Figure 18: FLIRT linear registration a) used to co-register axial non-MT PD-weighted image to axial T1-weighted image, and generate whole brain b), grey matter c) and white matter d) non-MT masks, for further generation of whole brain, grey matter and white matter MTR masks

f) In Jim 4.0, whole brain, grey matter and white matter masks were applied to the inherently coregistered MTR maps, producing images containing only parenchyma, white matter and grey matter MTRs.



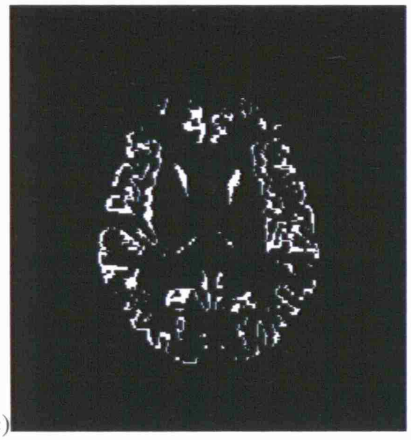
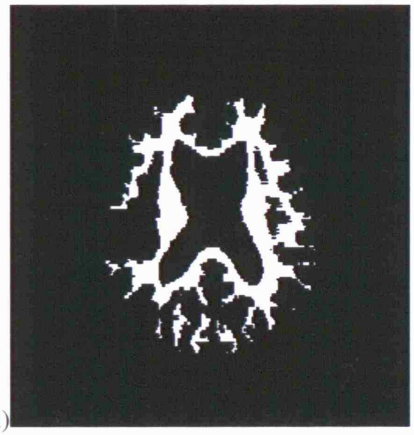
c)  d) 

Figure 19: Image masker a) used to generate whole brain b), grey matter c) and white matter d) MTR masks

g) An in-house MTR histogram generation algorithm was used to calculate MTR pixel intensity frequencies for each tissue segment. The histogram was divided into bins of 1 pu, to smooth out the effects of noise, while preserving the shape features of the histogram. Intersubject variability in specific tissue volumes was adjusted for by normalising the MTR histograms. This was achieved by dividing the number of counts in each sampling bin by the total number of pixels for each tissue segment. From each normalised histogram, the following histogram measures were calculated for whole brain, white matter and grey matter (Figure 20):

1. Average MTR (AVMTR)
2. Peak Height (PH)
3. Peak Location (PL)
4. MTR at 25^{th} percentile (MTR25%)
5. MTR at 50^{th} percentile (MTR50%)
6. MTR at 75^{th} percentile (MTR75%)

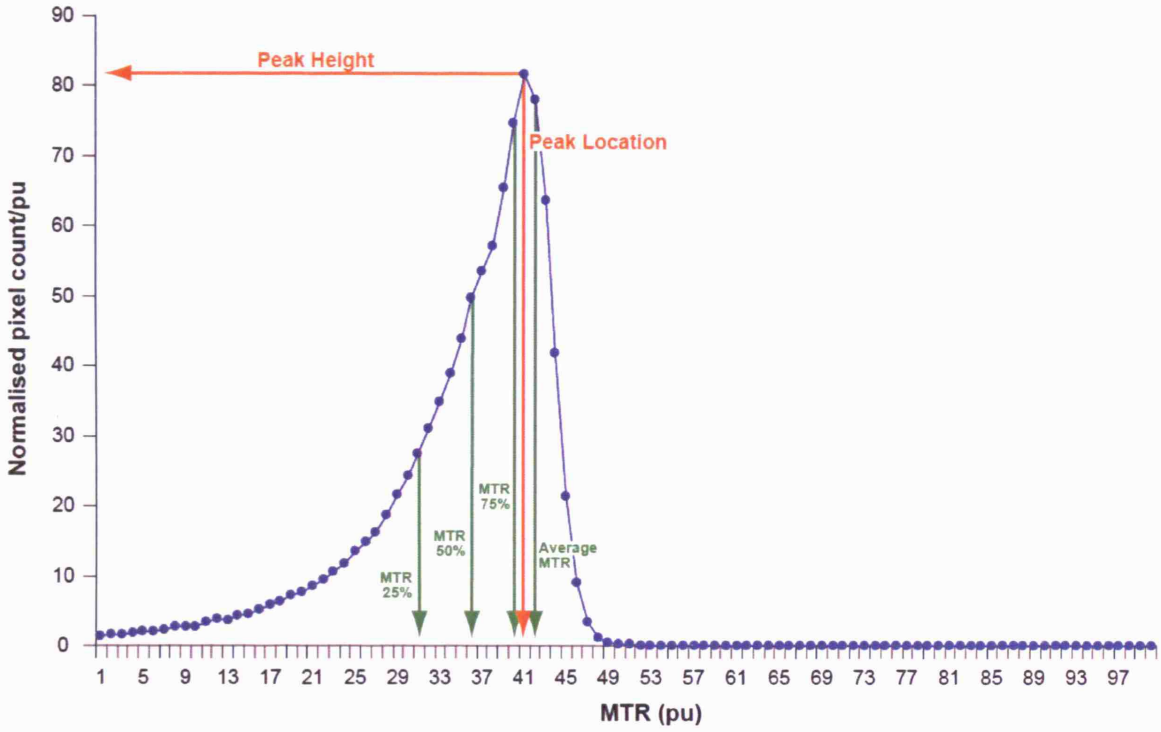


Figure 20: Diagrammatic representation of MTR histogram measures

2.3.3 Quality control

A physicist experienced in performing ROI and MTR histogram analysis and quality control of MT MRI scans reviewed the entire database of scans raw MTC datasets while blinded to all patient details, in order that images of inadequate quality could be excluded. Further quality control measures, outlined below, where undertaken throughout the data analysis.

It was necessary for data sets to meet the following criteria before being included in the subsequent analysis (see Figure 21 for examples):

1) Scan quality

- No deviations form the predefined study MT scan protocol (e.g. correct number of slices)
- No significant movement artefact (ghosting)
- Brain/CSF boundary definition adequate on all slices

- All intracerebral structures included (no scan cut off)

2) Quality of ROI and MTR histogram analysis

- No errors of inclusion of non-brain material and no errors of exclusion of brain material in the ROI analysis
- Adequate exclusion of non-brain material and visually correct anatomical segmentation for data set included in the MTR histogram analysis

3) Registration quality

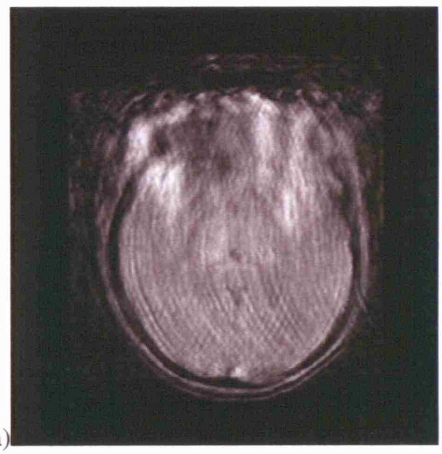
- Adequately accurate registration, as determined by visual inspection, between axial T1-weighted, non-MT, MT and MTR images for MTR histograms

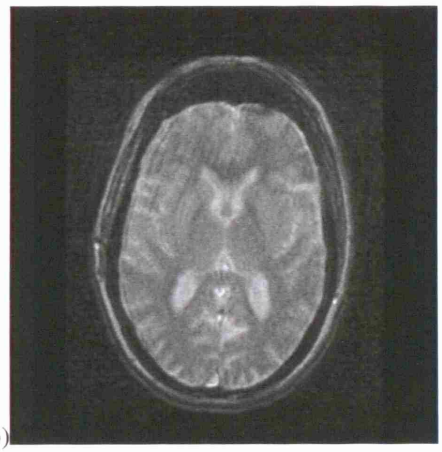
4) Intra-patient review

- Where baseline scan was of inadequate quality, the original baseline scan was excluded and a subsequent scan chosen for analysis (in 5 patients)

5) Singleton scans

- Patients with only one scan were excluded from longitudinal analysis, but included in baseline analysis

a) 

b) 

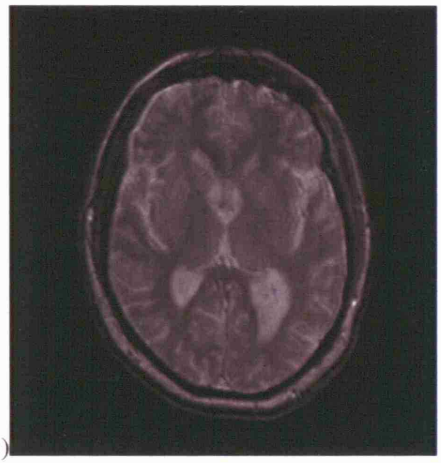
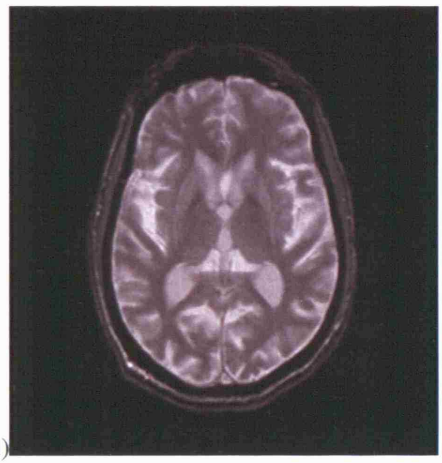
c)  d) 

Figure 21: Effects of motion on axial MT images a) Severe motion artefact in a patient with IPD with MMSE<10 b) Vacuum hood placement in a patient with IPD, MMSE 28, and impaired concentration c) Oral sedation leading to rebound agitation in a patient with sCJD d) Motion-free image upon GA in a patient with IPD, and MMSE 18

2.4 FLAIR/DWI analysis

A blinded, randomised assessment of signal abnormalities in the cerebral cortex and basal ganglia on standard clinical MR sequences, namely DWI and FLAIR (Figure 22) was also performed. These areas may show hyperintensity in all forms of human prion disease and this analysis was carried out to determine whether quantitative MT changes are observed in areas with or without signal change on conventional MRI. Two consultant neuroradiologists independently reviewed baseline and longitudinal PRION-1 scans separately with 2 research fellows (myself and an MRC Prion Unit fellow), whilst being blinded to patient details and diagnosis, and determined whether any signal abnormalities were observed bilaterally in:

a) Caudate nuclei

b) Putamina

c) Thalami (including pulvinar region)

d) Frontal cortices

e) Parietal cortices

f) Temporal cortices

g) Occipital cortices

h) Insular cortices

i) Cingulate cortices

A proforma was devised to record the above abnormalities (appendix F).

Wherever discrepancies were observed between the assessments from both radiologists a final opinion was reached by consensus.

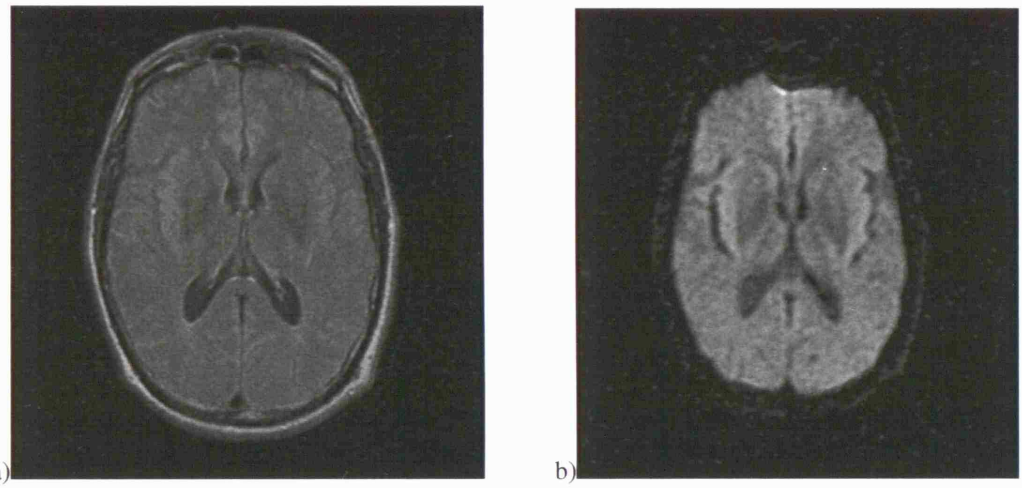


Figure 22: a) Axial FLAIR image and b) Axial DWI image (b=1000) from a symptomatic patient with inherited prion disease (P102L mutation)

2.5 PRION-1 TRIAL

Section 2.5 describes the design of PRION-1 Trial [5], the aim of this thesis as part of the trial, and clinical assessments carried out as part of the trial, which were incorporated in the analysis for this thesis.

2.5.1 Ethical approval

The PRION-1 trial was conducted throughout the UK with Ethical approval from the Eastern Multi Regional Ethics Committee (MREC) in March 2004.

2.5.2 Trial design and purpose

The PRION-1 trial was a partially-randomised patient-preference trial to evaluate the activity and safety of quinacrine in adults or children aged twelve years or more diagnosed with any type of human prion disease. At the design phase of the trial it was felt that in a uniformally fatal and rapidly progressive disease, very few patients would be willing to enter a randomized trial, so patients were placed into three groups according to preference and willingness to be randomised, but were followed up with the same schedule of evaluations:

1. Patients who were willing to be randomised into a controlled comparison of immediate open-label quinacrine versus no quinacrine with the option of starting open-label quinacrine after 6 months (deferred quinacrine).

2. Patients who preferred to receive quinacrine immediately.

3. Patients who preferred not to receive quinacrine.

This is summarised in Figure 23 [184].

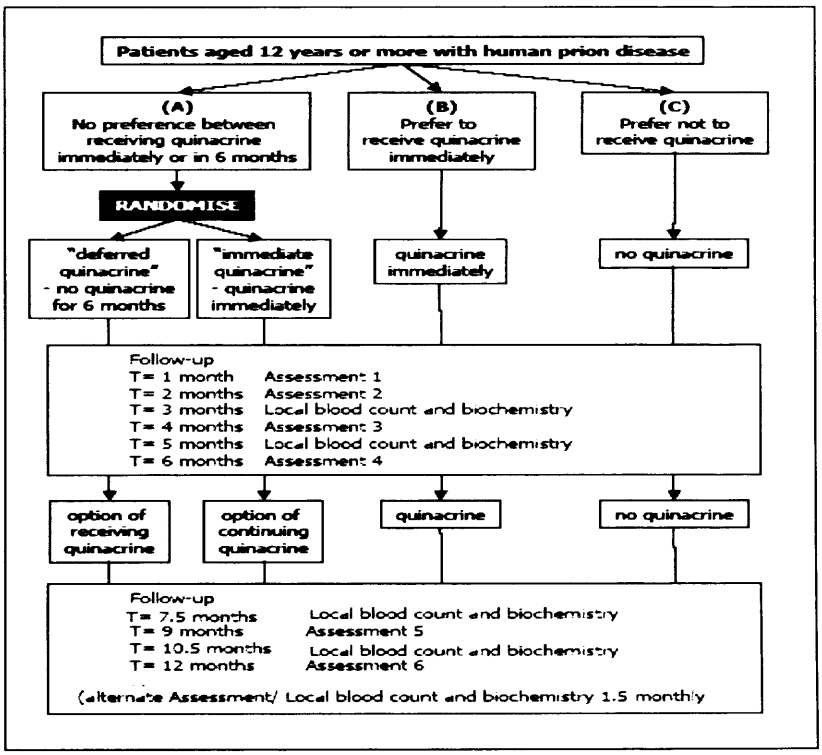


Figure 23: PRION-1 trial design and follow-up schedule

Eligible patients had no clinical or laboratory abnormalities contra-indicating use of quinacrine. Patients who were randomised to or chose immediate quinacrine were given a loading dose of 1 g (200mg 6-hourly) orally followed by 100mg of quinacrine three times a day.

PRION-1 was a 3 year trial. Recruitment into the trial took place over 2 years with follow up of 1 year after the last patient had been randomised or enrolled. It aimed to enrol and follow up 160 patients until the end of the trial, with 80 patients in the randomised arm. Lack of deterioration in the independently-rated digital video recording of the neurological exam was a primary efficacy endpoint. Secondary efficacy endpoints were neurological and neuropsychological changes, including changes in markers of disease activity, MRI, EEG and neurological assessments, where appropriate.

PRION-1 was the 1st UK clinical trial in human prion disease and aimed to establish an appropriate framework for the clinical assessment of therapeutic options for human prion disease that could be refined or expanded in the future, as new agents become available.

Prusiner and colleagues studied a range of drugs known to penetrate the central nervous system for their ability to inhibit PrP^{Sc} production in a murine scrapie cell culture model. They demonstrated in their report in 2001 [185] that relatively low concentrations of quinacrine (levels that might be realistically achieved in patients) were effective in this assay. As quinacrine could be taken orally, was known to cross the blood-brain barrier and there was considerable existing clinical experience of its consumption by humans as an anti-malarial and for long-term treatment of arthritis (without any common adverse effects), it was suggested as an immediate candidate

for the treatment of prion disease in humans given its safety profile, hence the rationale for its use in PRION-1.

PRION-1 is the 6th trial of quinacrine in human prion disease, and the 1st controlled clinical trial in a larger cohort of CJD patients. Four previous studies had between 1 and 4 patients [186, 187, 188, 189]; a French study of 35 severely affected patients with prion disease showed a slight, but non-statistically significant increase in survival duration [190]. A randomised trial comparing quinacrine, quinacrine plus chlorpromazine and placebo in human prion disease began in the United States in 2001. A randomised, placebo-controlled study from the same group investigating the efficacy of quinacrine in sporadic prion disease in over 100 patients began in March 2005. Results are awaited.

The key objectives of the PRION-1 trial were to determine whether quinacrine has a significant effect on cognition or survival duration in all types or particular sub-types of human prion disease. Fewer patients were recruited into PRION-1 than predicted and of those recruited only a quarter had MRI including MT sequences making them suitable for analysis and inclusion in this thesis. Only a few patients took quinacrine and no comparisons have been made between quinacrine treated and non-treated groups in this thesis, nor was it the main objective of this thesis to report the effects of quinacrine on those recruited in PRION-1. The therapeutic effects of quinacrine in patients recruited for the whole of PRION-1 are the subject of a separate investigation, underway at the Medical Research Council Clinical Trials Unit, London NW1 2DA.

The main aim of this thesis was to demonstrate and quantify associations between MT and clinical measures in human prion disease, thus it only includes data from those

patients taking part in the PRION-1 trial who underwent quantitative MT imaging and clinical assessments at, or near, the same time as MT MRI scans.

2.5.3 Clinical assessments

PRION-1 clinical assessments comprised videoed and non-videoed neurological examinations according to the assessment schedule given in Figure 10. Equal numbers of assessments were carried out either at the National Hospital for Neurology and Neurosurgery (NHNN), London, WC1N 3BG as inpatients or outpatients or on domiciliary visits at the patients' home, local hospital or hospice. These assessments took place throughout the UK and were carried out by the author and another trial clinician.

2.5.3.1 Non-videoed clinical examination

This included assessment of the following clinical scores. Score sheets may be found in Appendices G-L:

- *Mini Mental State Examination (MMSE)* [146]: used as an overall severity score for cognitive function. A maximum score of 30 indicates no deficit of memory or language and a minimum score of 0 indicates severe deficit of memory and language.
- *Clinician's Dementia Rating Scale (CDR)* [149]: a measure widely used in clinical studies of dementia. Using a semi-structured interview with the patient or a caregiver, it measures a patient's function in areas of memory, orientation, judgement and problem solving, community affairs, home and hobbies, and personal care, providing a score ranging from 0 to18. Each component is

scored from 0 to 3 (0=no impairment, 1=mild impairment, 2=moderate impairment, 3=severe impairment).

- *Rankin scale* [154]: a global assessment of the impact of disease on activities of daily living, providing a score ranging from 0 to 5 (0=no symptoms, 1=no significant disability despite symptoms, 2=slight disability, 3=moderate disability, 4=moderately severe disability, 5=severe disability).
- *Alzheimer's Disease Assessment Scale (ADAS-COG)* [141]: performed in those with a MMSE of 10 or greater. This is a more detailed assessment of cognition, validated in Alzheimer's disease. Score ranges from 0 to 75. It assesses 12 items covering word recall, naming objects and fingers, performing simple commands, constructional praxis, ideational praxis, orientation, word recognition, remembering test instructions, spoken language ability, word-finding difficulty, comprehension and concentration.
- *Glasgow Coma Score (GCS)* [191]: a measure of responsiveness in the most severely affected patients, with scores ranging from 3 to 15.
- *Barthel Activities of Daily Living scale (ADL)* [155]: measures ability to perform activities of daily living on a scale of 0 (unable to perform any ADL) to 20 (can perform all ADLs).
- *A clinician's global impression of disease severity (CGIS)* [192]: A scoring system completed by both doctors and nurses on the PRION-1 trial to assess change in severity of disease (0=normal, not ill, 1=borderline mentally ill, 3=mildly ill, 4=moderately ill, 5=markedly ill, 6=severely ill, 7=amongst the most ill). For the purpose of this thesis, assessments made by the doctors alone were included.

2.5.3.2 Videoed clinical examination

A digital visual recording of a standardised neurological examination and Brief Psychiatric Rating Scale (BPRS) was performed on all trial patients at each trial assessment.

2.5.3.2a Standardised Neurological examination

Clinical examination consisted of a formalised examination based on the Queen Square format, summarised on a standardised neurological examination form (see Appendix M). There were three sections, a 15 point cognitive, 11 point motor and an overall assessment.

The cognitive tests comprised:

1) Memory task – patients were asked their name, age, and either the month of their birthday or if they were married.
2) Letter cancelling task – patients were asked to identify all occurrences of a designated letter in a grid of different letters
3) Line drawing task – ten drawings of common objects were printed on a page, and the patient was asked to name them
4) Reading task – one of three paragraphs was placed in front of the patient who was asked to read the passage, and remember as much as they could.
5) Spelling task – the patient was asked to spell six designated common words
6) Fragmented letters task – the patient was asked to identify three letters that had been fragmented
7) Fragmented Objects task – four line drawings of objects, with some elements of the object removed were asked to be identified
8) Calculation talk – four increasingly difficult addition tasks

9) Miming task – patients were asked to show how they would brush their teeth, comb their hair and use a screwdriver
10) Copying Gestures – patients were asked to copy three hand gestures
11) Frontal lobe sequencing task – alternating hand movements were copied
12) Words beginning with a letter task – patients were asked to name as many words beginning with a particular letter as they could
13) Proverbs – interpretation of two common proverbs
14) Digit span – 3 to 7 number digit span was tested
15) Recall – recall of the passage read out previously was tested. Examples of picture tests are given in Appendix H.

The motor tests comprised:

1) Eye movements – full range of eye movements was tested, as well as pursuit
2) Finger nose testing – bilateral finger nose testing
3) Rapid alternating task – patient tapped front and back of hand on opposite palm, performed bilaterally
4) Sequential index finger tapping – performed bilaterally
5) Sequential opposition – thumb opposed to each of the fingers in turn and repeated bilaterally
6) Primitive reflexes – glabellar tap, pout reflex and palmomental reflex were tested
7) One minute observation – all four limbs observed for one minute for abnormal movements
8) Walking– asked to walk length of room
9) Heel toe walking –asked to walk heel to toe for length of room, unsupported
10) Romberg's

11) Neurological examination - tone, power and reflexes were assessed bilaterally

The overall assessment section asked whether the patient was able to cope with task demands, whether the overall level of attention and concentration was satisfactory and if the patient was cooperative. Overall impression of cognitive, extrapyramidal, pyramidal and cerebellar impairment was then assessed. This thesis examines the clinical scores from this assessment:

1. Cognitive impairment (0 (none), 1 (mild), 2 (moderate), 3 (severe), 4 (cannot assess))
2. Extrapyramidal impairment (0 (none), 1 (mild), 2 (moderate), 3 (severe), 4 (cannot assess))
3. Pyramidal impairment (0 (none), 1 (mild), 2 (moderate), 3 (severe), 4 (cannot assess))
4. Cerebellar impairment (0 (none), 1 (mild), 2 (moderate), 3 (severe), 4 (cannot assess))

In order to minimise learning effect, 3 versions of each of the 5 picture tasks test were developed and presented at random to the patient. Each test was scored individually during the assessment. To ensure that the neurological rating scales were applied consistently, they were always carried out by 1 of the 2 PRION-1 trial clinicians (myself and a clinical fellow), and videoed by 1 of the trial nurses.

2.5.3.2b Severely Affected Protocol (SAP)

A truncated version of the standardised neurological examination was performed for severely affected patients, defined as those who scored 10 or less on the MMSE, or had a GCS of less than 15. These tests included the memory test, eye movements,

primitive reflexes, 1-minute observation, walking and neurological examination and are starred on Appendix N (Neurological Examination Form).

2.5.3.2c Storyboard

Filming of the examination was performed according to a standard storyboard (Figure 24), which defined the camera and shot that should be used to capture the response in each test. Included in the storyboard was a label shot at the beginning of the examination for each camera giving the patient's initials, date of birth and date of assessment.

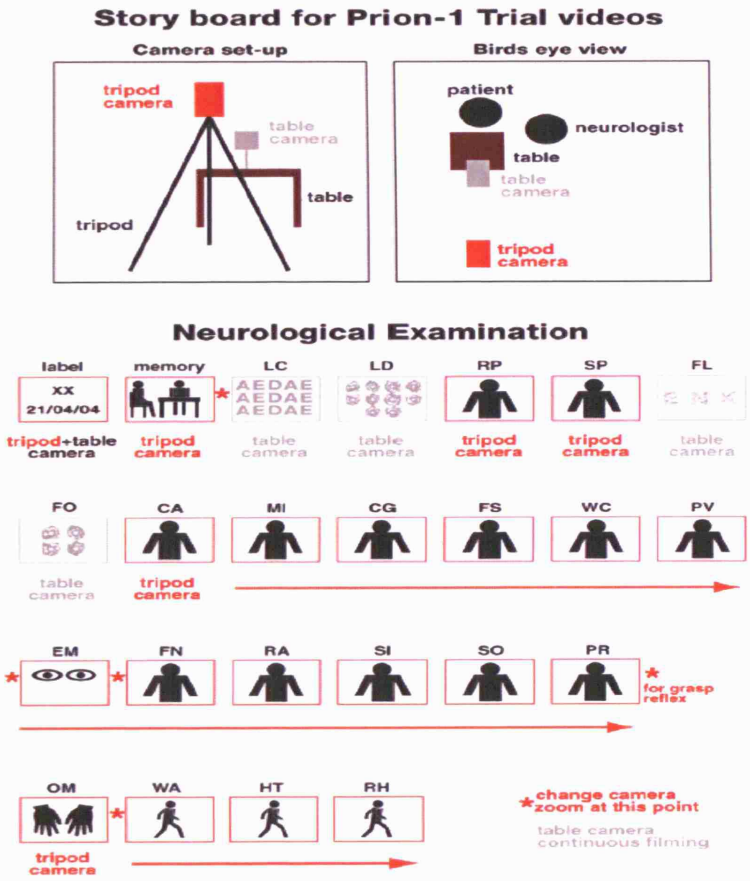


Figure 24: Story board for PRION-1 trial video of standardised neurological examination

2.5.3.2d Equipment

Two digital camcorders were used to film each examination. One was placed on a tripod at shoulder height, and the other clamped to the table in front of the patient to capture responses to the tabletop cognitive tests and for better sound recording. Mini-DV format cameras were used, each Mini-DV tape was labelled with the trial study number of the patient and week of assessment.

2.5.3.2e Post acquisition processing

After each video was recorded the labelled tape was stored in a labelled box in the National Prion Clinic (NPC) offices. Each visual recording was then allocated a randomised video identification number by an MRC Prion Unit research fellow, who further processed the tapes for the purpose of independent assessment. Digital tapes were edited using *Adobe Premiere Pro*™ video editing software. Each edited visual recording was assembled into a set order of examinations, starting with a disclaimer notice, and with the video identifier number displayed at all times. Each clip was labelled and shown in the specified order, separated by five seconds of blank video. A disclaimer notice was shown at the end. If a severely affected protocol was used, this was labelled at the start. All DVDs were stored in a fireproof safe located at the NPC.

2.5.3.2f Independent Assessment

The purpose of digitally recording the neurological assessment was to allow subsequent blinded scoring by an independent assessor. Independent assessment of the neurological examination and the BPRS assessment were carried out by a specified independent neurologist and psychiatrist. The independent neurologist or psychiatrist was given a portable DVD player with a yellow filter over the screen to

blind them to quinacrine usage (quinacrine is a dye and can turn the skin yellow). For each visual recording, a scoresheet was filled out (see Appendix O), the only identifier being the video identification number. Completed forms were stored in a fire safe at the NPC.

2.5.3.2g Brief Psychiatric Rating Scale (BPRS) [152]

Psychiatric status was initially assessed by one of the two trial clinicians, using a series of standardised structured questions. This was followed by an independent consultant psychiatrist using the videoed BPRS, providing a final score ranging from 24 to 168 (see form in Appendix P). The BPRS scored 24 items on a qualitative scale from 1 to 7 (according to whether the symptom was absent, very mild, mild, moderate, moderately severe, severe or extremely severe). Components included somatic concern, anxiety, depression, suicidality, guilt, hostility, elated mood, grandiosity, suspiciousness, hallucinations, unusual thought content, bizarre behaviour, self-neglect, disorientation, conceptual disorganisation, blunted affect, emotional withdrawal, motor retardation, tension, uncooperativeness, excitement distractibility, motor hyperactivity, mannerisms and posturing, anxiety, depression, hostility, disorientation and blunted affect. Human prion disease is associated with complex neuropsychiatric manifestations and quinacrine may also rarely cause psychosis. Though independent assessment of the BPRS videos could not be completed in time for this thesis, scores derived from assessment of trial clinicians were used for statistical analysis of data.

2.6 STATISTICAL ANALYSIS

This section describes the methods adopted for statistical analysis of PRION-1 data.

Each of the global and regional MT measures, and clinical measures were analysed following the same structured plan, as outlined below.

For the purposes of the analyses of MT and clinical measures, baseline was defined as the date of the first MT MRI scan within PRION-1 which was of acceptable quality after the independent quality control process. This was because it was not possible to scan all patients at their first study visit, some patients enrolled in PRION-1 at various disease stages and some scans were discarded due to quality control issues, as discussed in detail later in the results section.

Time was measured in months from this first scan date and exact timings were used instead of the nominal visit months.

2.6.1 Univariate relationship between MT measures and clinical scores at baseline

Bivariate Spearman rank correlation analysis was performed between baseline MT measures and videoed and non-videoed clinical scores, since the data was not completely normally distributed. This analysis determines if baseline MT measures are associated with baseline clinical scores, that is whether patients with low MT measures also have poorer clinical ratings at baseline.

2.6.2 Independent relationships between baseline MT measures and other baseline factors

The next question to be addressed was whether relationships identified in analysis 1 held independently, that is which clinical and other baseline factors were independently associated with baseline MT measures as the primary outcome.

a) Baseline factors which were considered included sex, age at first visit, duration of symptoms at baseline visit, disease type and scores from neurological and psychiatric

examination (BPRS, GCS, Barthel ADL, MMSE, ADAS-COG, CDR, Rankin, CGIS, and videoed cognitive and motor scores). The nearest measurement within 7 days on either side of the baseline visit was used.

b) Baseline MT measures were checked for approximate normality and if not, log transformation was considered to achieve approximate normality.

c) Standard linear regression models were used, with the baseline MT measures as the dependent variable, considering each of the factors listed in (a) one at a time (univariable analysis). Both the statistical significance and the size of the estimated effect of the baseline factor on baseline MT measures were considered.

d) Any baseline factors which reached a relatively generous significance threshold ($p<0.10$), to take into account the relatively low power of the analysis due to the small number of observations, were jointly considered in a multivariable model with outcome measure baseline MT measures. Backwards elimination with exit probability $p=0.05$ was used to identify which of the baseline factors were independent predictors of baseline MT measures as opposed to potential confounders.

2.6.3 Relationship between decline in MT measures and baseline factors

The third question which was addressed was how decline in MT measures over time were associated with clinical scores measured at the baseline visit: the aim was to identify independent baseline predictors of subsequent decline in MT measures. Any such measures could potentially be used to predict future outcome.

a) Rate of decline in MT measures was estimated for each patient (as measured by the B value or slope) based on a linear regression model including all MT measures for that patient (outcome variable) and time (measured in months from baseline visit) as an explanatory variable. The possibility of non-linearity in MT measures and clinical

measures was considered graphically but observations were too few to explore robustly.

b) It was determined whether there was a significant decline in MT measures for each patient. This was assessed by considering the statistical significance of the linear trend calculated in (a). A one-sample t-test was also used to assess the overall decline in MT measures in the group of patients as a whole, that is, to determine whether decline was significantly different from zero.

c) Model fitting followed the same procedure as for (2) above, but using the estimated decline (B value) as the outcome variable (standard normal linear regression) and each baseline factor as an explanatory variable.

2.6.4 Relationship between decline in MT measures and decline in other factors

The fourth question which was addressed was how decline in MT measures over time was associated with decline in other factors measured over the same period as the MT measures. The aim was to investigate whether changes in MT measures were paralleled by changes in neurological and psychiatric rating scales.

a) Linear decline in each score from neurological and psychiatric examination (BPRS, GCS, Barthel ADL, Rankin, MMSE, ADAS-COG, CDR, Rankin, CGIS and videoed cognitive and motor scores) over the time period in which MT measures were measured was estimated as in (3a) above.

b) A one-sample t-test was also used to assess the overall decline in clinical scores in the group of patients as a whole, that is, to determine whether decline was significantly different from zero.

c) Scatterplots of estimated decline (B values) in MT measures against estimated decline in each of the other factors were compared. Statistical significance was

assessed using Spearman's rank correlation coefficient (as data was not normally distributed).

3. RESULTS

3.1 DESCRIPTION OF PRION-1 MT MRI DATABASE

3.1.1 Pre-quality control

The entire database of PRION-1 MT MRI scans is first described, prior to quality assessment. 115 MT MRI scans were performed in 30 patients with any form of prion disease. Each patient had between 1 and 10 scans each (mean 5.50). The earliest scan was in August 2004, the latest in February 2007. The number of MT MRI scans performed in each PRION-1 patient prior to assessment of the images for data control purposes is shown in Figure 25.

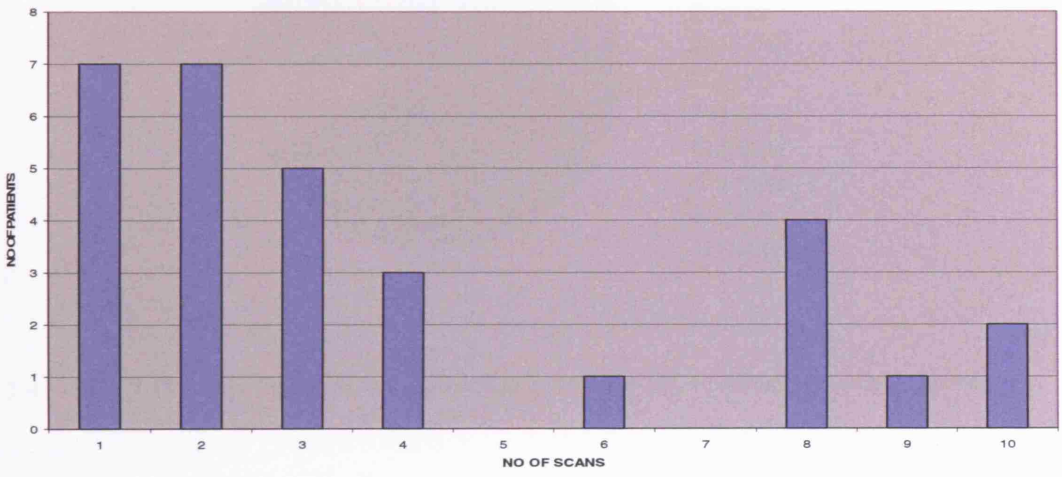


Figure 25: Number of patients undergoing specific numbers of MT MRI examinations prior to data quality assessment

The profile of different types of prion disease within the full scan database is shown in Figures 26 a and b:

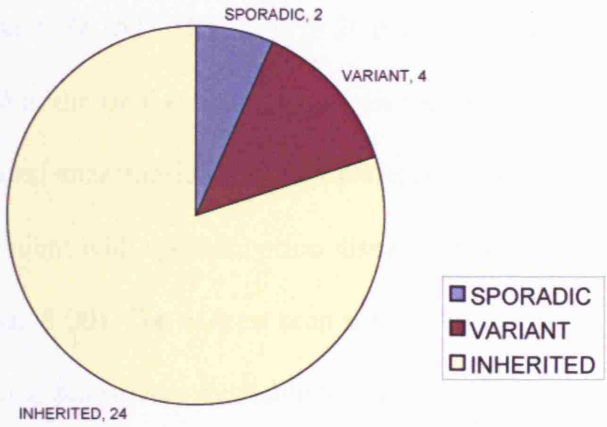


Figure 26a: Pie chart showing types of prion disease in 30 patients pre-quality assessment

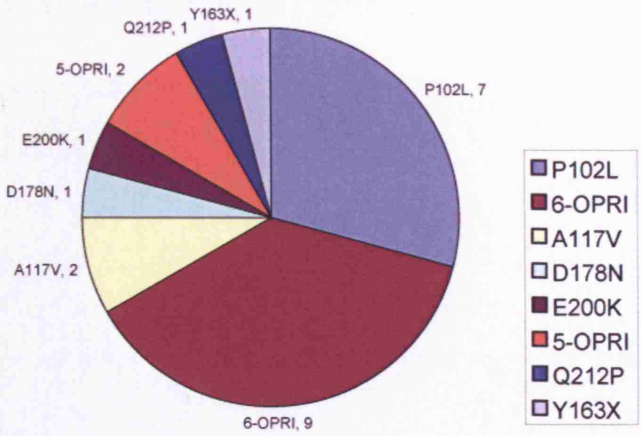


Figure 26b: Pie chart showing types of inherited prion disease in 24 patients pre-quality assessment

3.1.2 Quality control

27 MT scans were discarded during the quality control process due to:

1. Motion artefact - 25 scans in 14 patients with inherited, sporadic and variant prion disease

2. Incorrect number of slices - 2 scans in 2 patients with inherited prion disease

3.1.3 Post-quality control

After quality assessment, 88 MT MRI scans in 26 patients with any form of prion disease were included in the final statistical analysis for this thesis. 19 scans were performed under general anaesthesia (GA) in 7 patients (6 patients with inherited prion disease and 1 patient with sporadic prion disease). Each patient had between 1 and 9 scans each (mean 5.00). The earliest scan was in August 2004, the latest in February 2007. Baseline scans were available for all 26 patients. Longitudinal scans were available for 20 patients (19 patients with inherited prion disease and 1 patient with sporadic prion disease). The number of MT MRI scans in each PRION-1 patient after quality assessment, is shown in Figure 27.

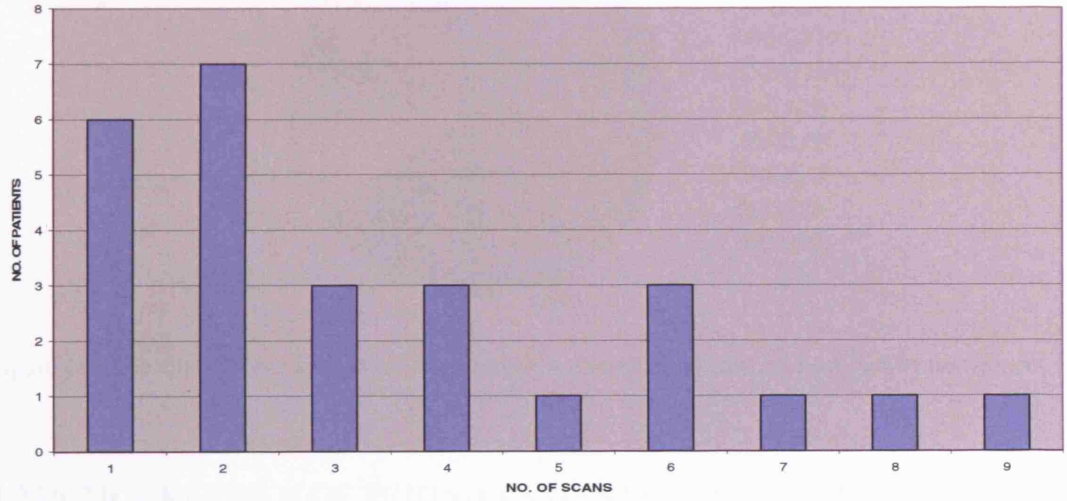


Figure 27: Number of patients for whom specific numbers of MT MRI examinations remained for analysis after image quality control process

The profile of different types of prion disease after quality control process is described in Figures 28 a and b:

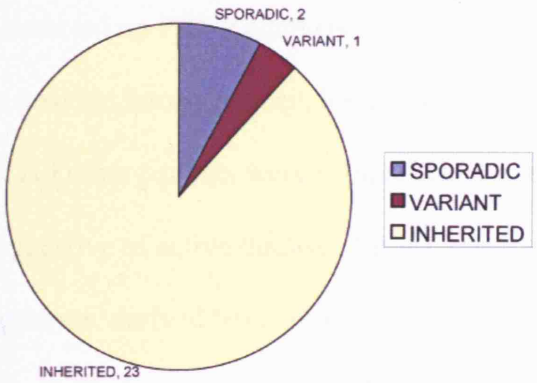


Figure 28a: Pie chart showing types of prion disease in 26 patients post-quality assessment

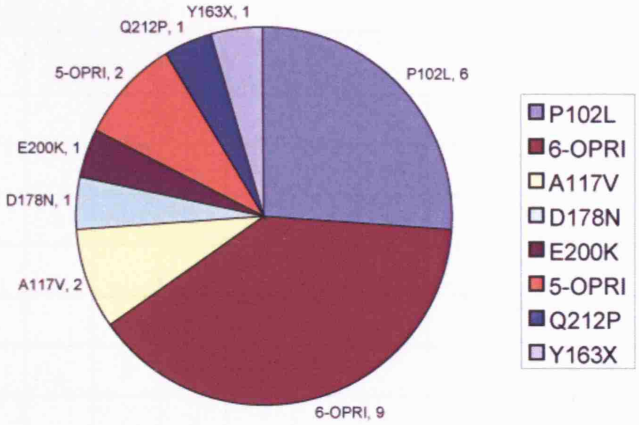


Figure 28b: Pie chart showing types of inherited prion disease in 23 patients post-quality assessment

3.2 DEMOGRAPHICS OF PRION-1 PATIENTS IN THIS THESIS

26 patients (14 male, 12 female, mean age 48.5 years, range 32-65 years) with inherited, sporadic and variant forms of human prion disease, recruited into the MRC PRION-1 trial from the National Prion Clinic, were included in the final analysis for this thesis (though prior to quality control there were 30 patients, but scans from 4 patients were discarded). 20 patients were symptomatic and 6 were asymptomatic. 2 of the patient group had sCJD, 1 had vCJD and PRNP analysis confirmed inherited prion disease in 23 patients, with P102L, 6 OPRI, A117V, 5 octapeptide repeat insertions (5 OPRI), Q212P, Y163X, E200K and D178N mutations. 6/23 InhPrD patients were asymptomatic, with P102L, D178N, E200K, A117V and 5 octapeptide

repeat insertions (5 OPRI) detected on PRNP analysis, carried out at a pre-symptomatic stage due to a positive family history, but no detectable signs or symptoms of prion disease. All other patients were symptomatic, with clinical or investigative parameters suggestive of active disease. Table 6 describes the demographics of these 26 patients, derived from a total cohort of 108 patients (62 sporadics, 9 variants, 37 inherited) recruited into the MRC Prion-1 Trial.

Table 6: Demographics of PRION-1 patients

No.	**Type**	**Follow-up in months**	**Age in years**							**Sex**	**Symptomatic**	**Quinacrine**
			30-34	35-39	40-44	45-49	50-54	55-59	60+			
1	sCJD	8							x	F	Yes	Yes
2	A117V	0			x					M	Yes	Yes
3	vCJD	0	x							M	Yes	Yes
4	P102L	0			x					F	No	No
5	P102L	17		x						M	No	No
6	Q212P	13		x						F	Yes	Yes
7	6 OPRI	25			x					M	Yes	Yes
8	6 OPRI	20			x					F	Yes	Yes
9	E200K	11				x				M	No	No
10	Y163X	3					x			F	Yes	Yes
11	6 OPRI	26			x					M	Yes	Yes
12	6 OPRI	2	x							F	Yes	No
13	6 OPRI	29	x							F	Yes	Yes
14	P102L	17						x		M	Yes	Yes
15	D178N	18		x						M	No	Yes
16	sCJD	0					x			M	Yes	No
17	6 OPRI	12		x						M	Yes	Yes
18	6 OPRI	0			x					F	Yes	Yes
19	P102L	4						x		M	Yes	Yes
20	P102L	20				x				M	Yes	No
21	5 OPRI	13			x					F	Yes	No
22	P102L	18						x		M	Yes	Yes
23	5 OPRI	7		x						F	No	No

24	6 OPRI	0			x					M	Yes	No
25	6 OPRI	3	x							F	Yes	Yes
26	A117V	12	x							F	No	No

3.2.1 Description of Quinacrine profile in patients in this thesis

A description of the quinacrine profile in patients included in this thesis is given below. Two patients chose to be randomised to immediate or delayed quinacrine, the rest chose immediate quinacrine.

16/26 PRION-1 trial patients included in this thesis took quinacrine. 7 were 6 OPRI patients, 3 P102L, 1 A117V, 1 D178N, 1 Y163X, 1 Q212 P, 1 sCJD and 1 vCJD. 6 patients started quinacrine during the pilot phase and then crossed over from pilot to full phase on quinacrine; 10 started in the full phase of the trial. All started on a dose of 300mg/day and all had time-matched MRI scans.

Two patients stayed on 300mg/day throughout the trial or until death. Eleven patients stopped quinacrine completely during the trial (4 voluntarily, 3 due to skin rashes, 2 developed nausea, 1 liver function test abnormality and 1 due to prion disease progression). Three patients had dose reductions but remained on quinacrine.

The mean length of treatment was 335 days (range 16-654).

The aims of this thesis did not include the determination of the effects, if any, of quinacrine on human prion disease, either clinically or on change in MTR. The thesis would however not be complete without a description of the profile of those who were on quinacrine. The effects of quinacrine are not known. This study includes a heterogenous group of patients and there is no evidence to suggest that quinacrine usage is likely to affect the relationship between MTR and clinical impairment. Quinacrine (Mepacrine hydrochloride) is known to penetrate the CNS and acts as a stimulant. There is no basis for assuming that quinacrine would be more or less

effective for any subtype of prion disease as no studies with human prion strains, human cell lines, or transgenic mice expressing human prion proteins are available. The only factor common to the cohort taking quinacrine in the PRION-1 trial is the starting dose. The disease types, number in each disease type, length of quinacrine usage, dosage profile and reasons for stopping were all different. No adjustments will be made to the data to allow for any potential quinacrine effects.

3.3 DESCRIPTION OF DATABASE OF VIDEOED AND NON-VIDEOED CLINICAL SCORES

115 videoed (cognitive impairment, extrapyramidal impairment, pyramidal impairment, cerebellar impairment, BPRS) and non-videoed clinical assessments (MMSE, CDR, ADL, ADAS-COG, GCS, Rankin, CGIS) were performed in patients with all forms of prion disease. Each patient underwent between 1 and 10 assessments each (mean 5.50). The earliest assessment was in August 2004, the latest in February 2007. 27 assessments were discarded together with corresponding MT scans during quality control.

Data from 88 assessments was used in this thesis, where temporally corresponding PRION-1 MRI scans were available. 12 patients were assessed according to a severely affected protocol (SAP) for the videoed neurological examination, where MMSE was less than or equal to 10, or was unrecordable. Each patient had between 1 and 9 assessments each (mean 5). The earliest assessment was in August 2004, the latest in February 2007. All patients had clinical assessments on the same day, or one day before or after PRION-1 scan, except one inherited patient (patient 12) who had their assessment 21 days before the scan, and one vCJD patient (patient 3), who had their assessment 28 days before the scan.

Baseline assessments were made for all 26 patients (23 inherited, 2 sporadic and 1 with variant form of prion disease). Longitudinal assessments were made for 20 patients (19 patients with inherited prion disease and 1 patient with sporadic prion disease). The minimum time interval between assessments was 2 months and the maximum time interval was 29 months in patients with inherited prion disease. In the sCJD patient (patient 1) the time interval between the first and last assessments was 8 months.

Figure 29 shows the percentage of missing values for each clinical score.

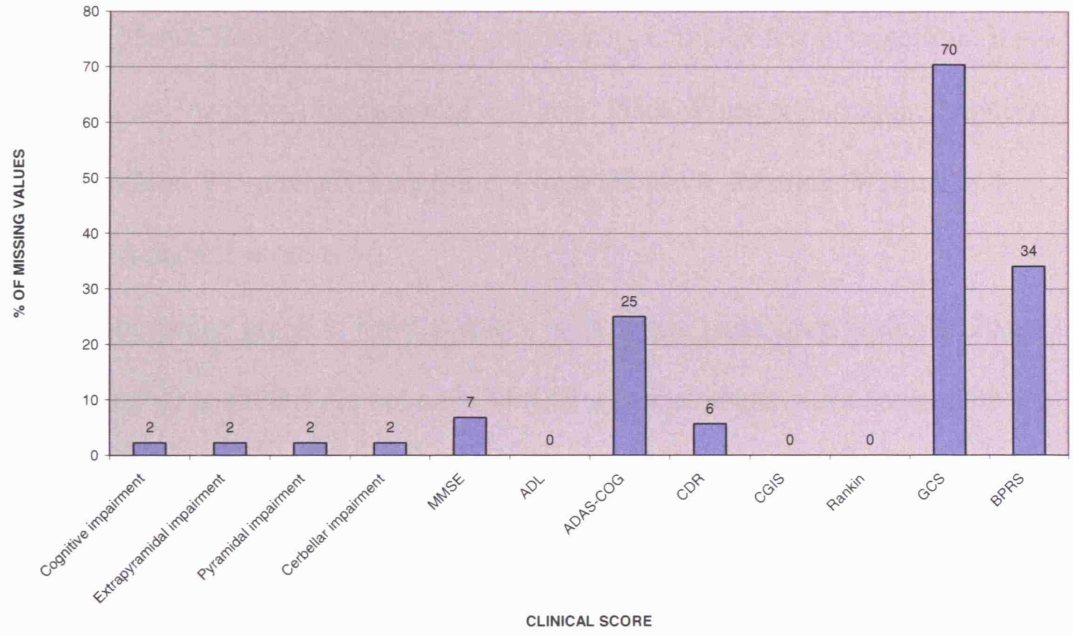


Figure 29: Percentage of missing values for each clinical score

The reasons for missing values were:

1. 2 lost videos (missing cognitive impairment, extrapyramidal impairment, pyramidal impairment, cerebellar impairment scores in 2 patients)

2. MMSE not recordable in severely affected, disorientated or dysphasic patients (6 assessments in 2 patients)

3. ADAS-COG not recordable in severely affected patients (12 assessments in 6 patients) or patients declined (10 assessments in 5 patients)

4. CDR not recorded as patient not orientated in person (5 assessments in 2 patients)

5. GCS was not recorded as MMSE was performed instead (12 assessments in 9 patients), or by error of omission for various reasons (50 assessments in 17 patients)

6. BPRS was not recorded in patients with severely affected protocol (12 assessments in 6 patients) or patients declined (18 assessments in 9 patients)

3.3.1 Longitudinal and baseline assessment of non-videoed clinical scores

3.3.1.1 MMSE

The Mini Mental Sate Examination is a moderately complex test of cognition. It is a 30 point score incorporating questions on Time, Place, Word registration, Attention and calculation, Word recall, Repetition, Comprehension, Reading, Writing and Drawing. A normal score is 30.

Firstly a composite graph of MMSE scores in each trial participant is shown. Patient 12 with InhPrD (6 OPRI) did not have MMSE recorded at any stage because of expressive dysphasia and a severely affected protocol was used for clinical assessment.

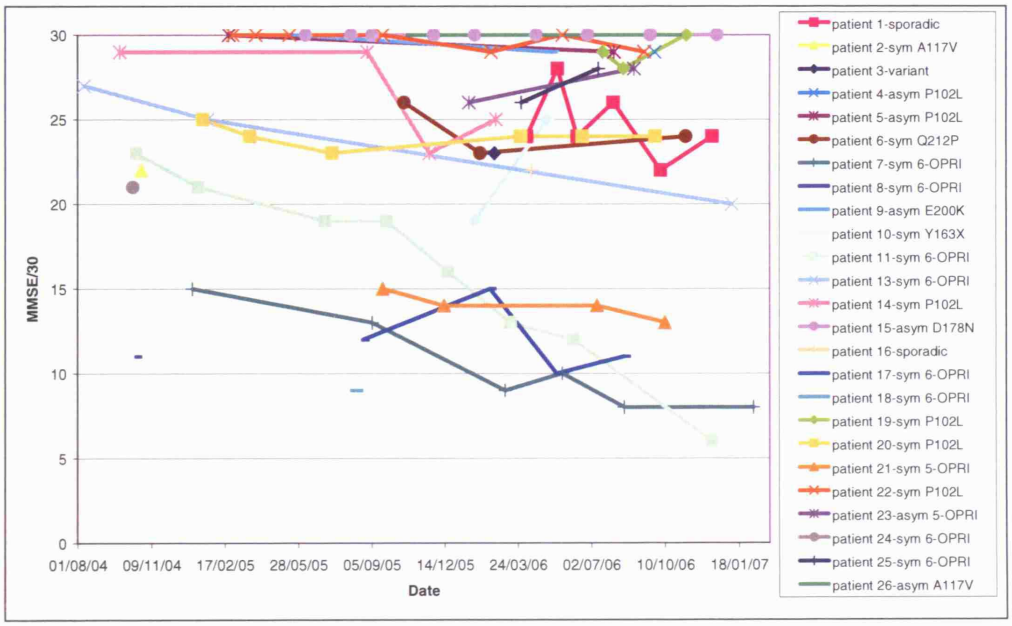


Figure 30: Composite graph of MMSE scores in each patient. MMSE fluctuates but tends to decline in these patients

Graphs for the patients in each group are shown below for ease of comparison, and comparisons are made with serial changes in MTR histogram and ROI measures.

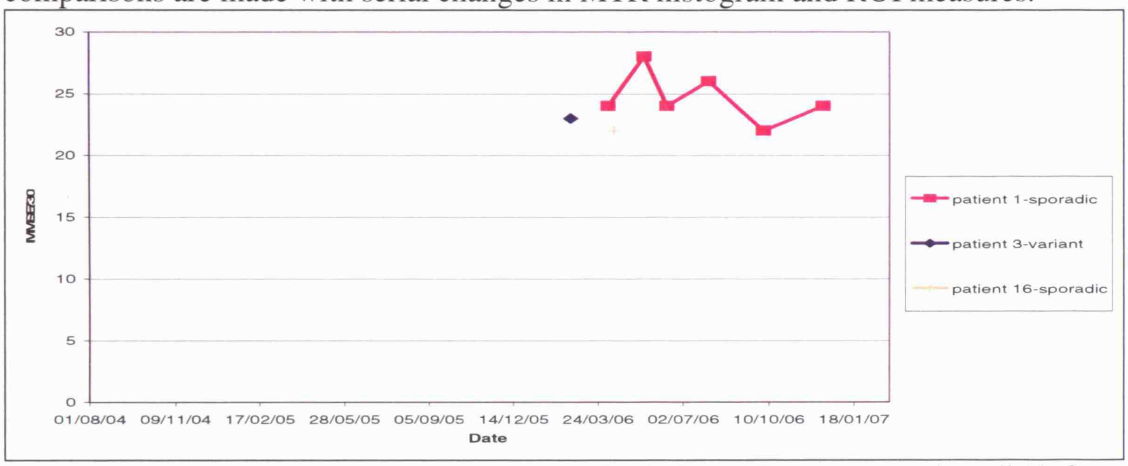


Figure 31: MMSE in sporadic and variant patients. Longitudinal MMSE scores were only available for patient 1 with sCJD, and showed a gradual decline over a period of eight months. MMSE at baseline for patient 16 with sCJD was 22, and for patient 3 with vCJD was 23

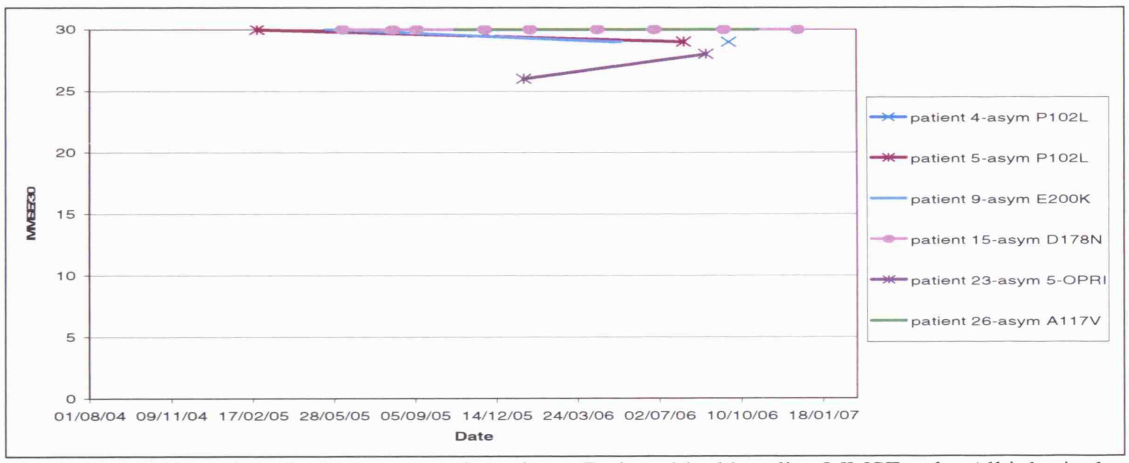
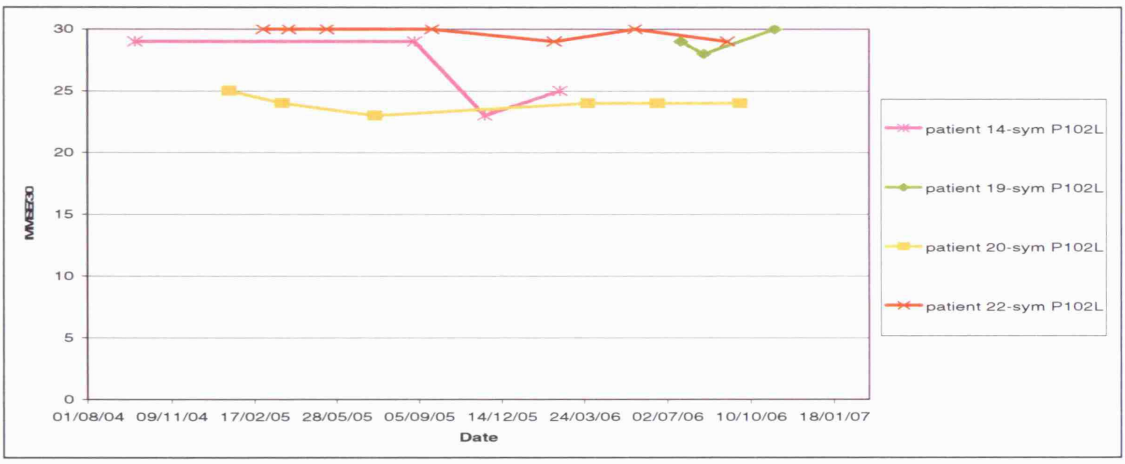


Figure 32: MMSE in inherited asymptomatic patients. Patient 4 had baseline MMSE only. All inherited asymptomatic patients scored full, or near full marks on MMSE except patient 23, who is dyslexic since childhood and had difficulty in subtracting serial 7s. Patients 5 and 9 dropped one point from a normal score of 30 (allowable in the normal population). Patient 23's MMSE was between 26 to 28


30
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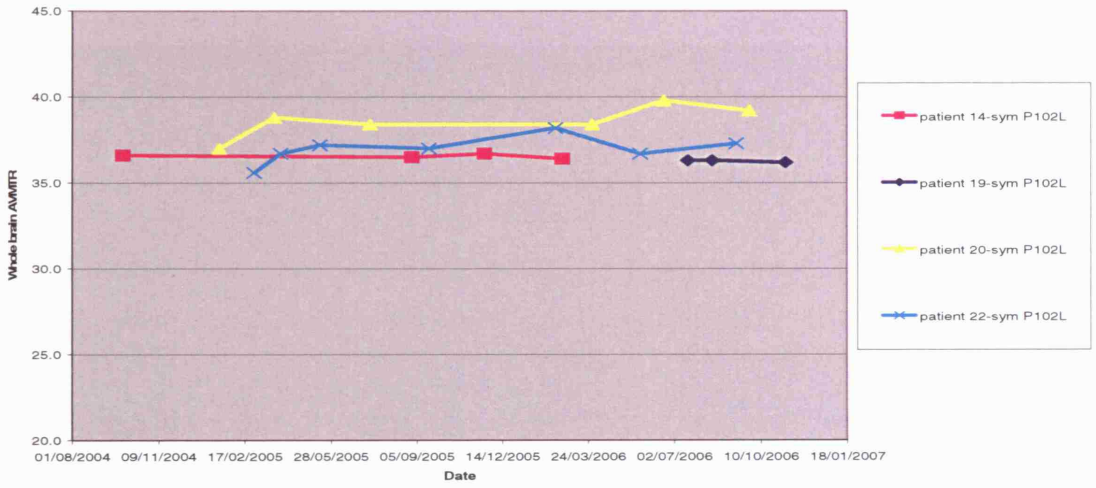
5
0
MMSE30
01/08/04 09/11/04 17/02/05 28/05/05 05/09/05 14/12/05 24/03/06 02/07/06 10/10/06 18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

a)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Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

b)


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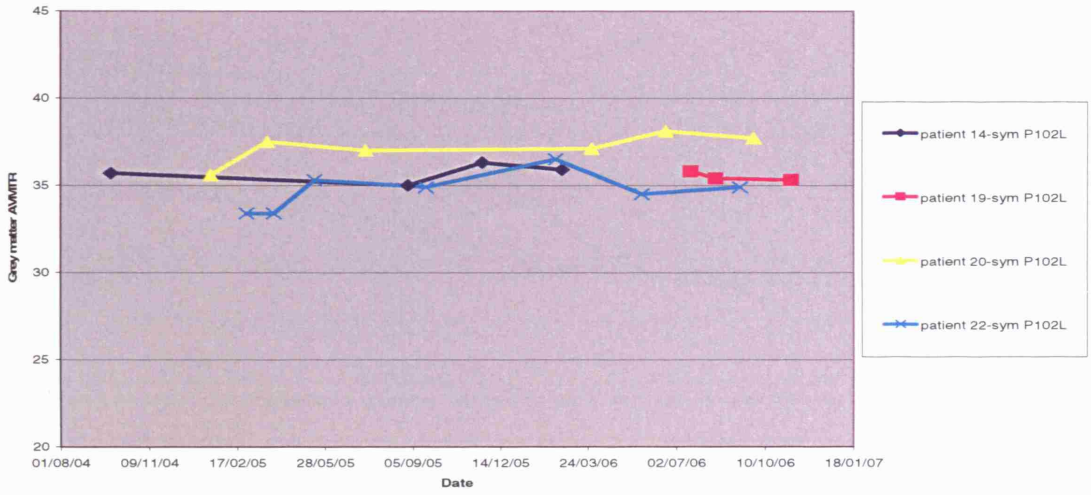
patient 20-sym P102L
patient 22-sym P102L

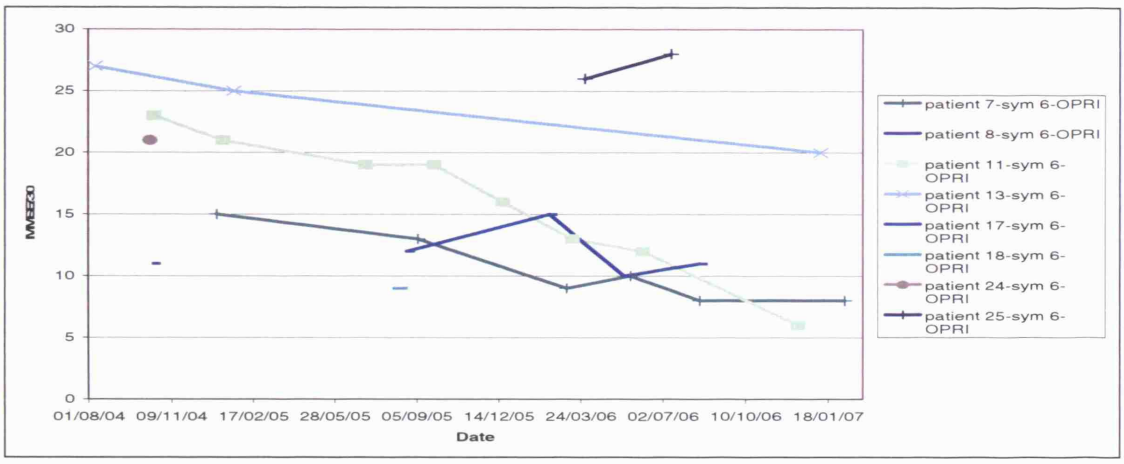
c)



d)

e)

Figure 33: a) MMSE in inherited symptomatic patients with P102L mutation. MMSE has remained stable in these patients, except in patient 14, where it has declined from 29 to 25 over a period of 17 months, the last assessment being 5 months before their death. In patient 20, it has remained stable at a lower value, ranging between 23-25 over a period of 20 months. b, c, d, e) Whole brain, white matter and grey matter AVMTR, and R caudate mean MTR in patients with P102L mutation either remains steady, or is gradually returning to baseline after an initial rise. A decline in R caudate MTR is seen in patient 19 only. Longitudinal changes in remaining MTR ROI and histogram measures are described in Appendix Q.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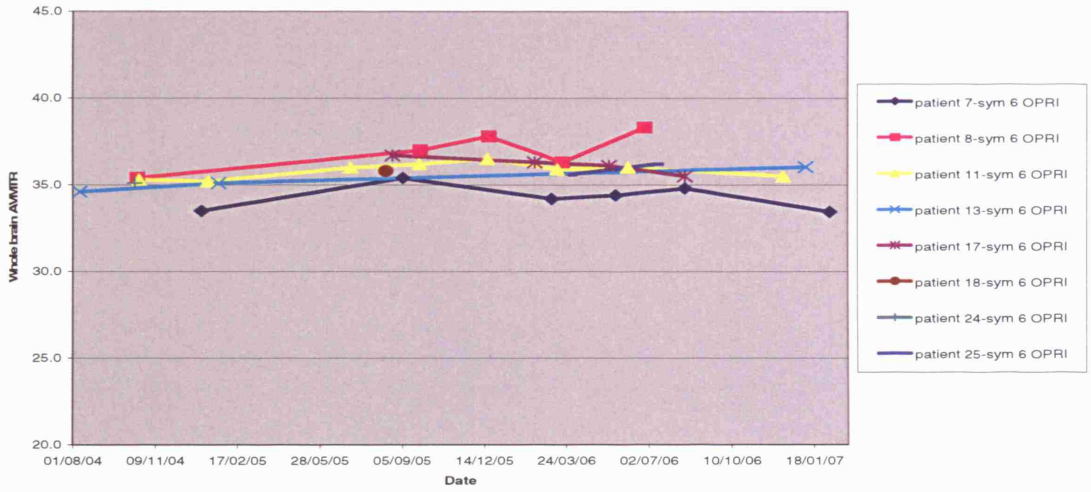
Date
patient 7-sym 6-OPRI
patient 8-sym 6-OPRI
patient 11-sym 6-OPRI
patient 13-sym 6-OPRI
patient 17-sym 6-OPRI
patient 18-sym 6-OPRI
patient 24-sym 6-OPRI
patient 25-sym 6-OPRI

a)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Whole brain AVMTR
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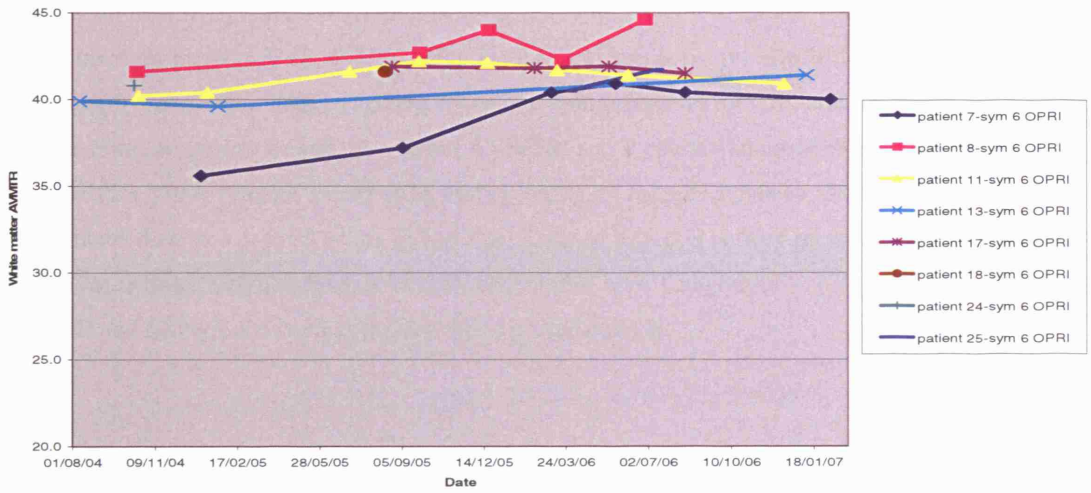
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

b)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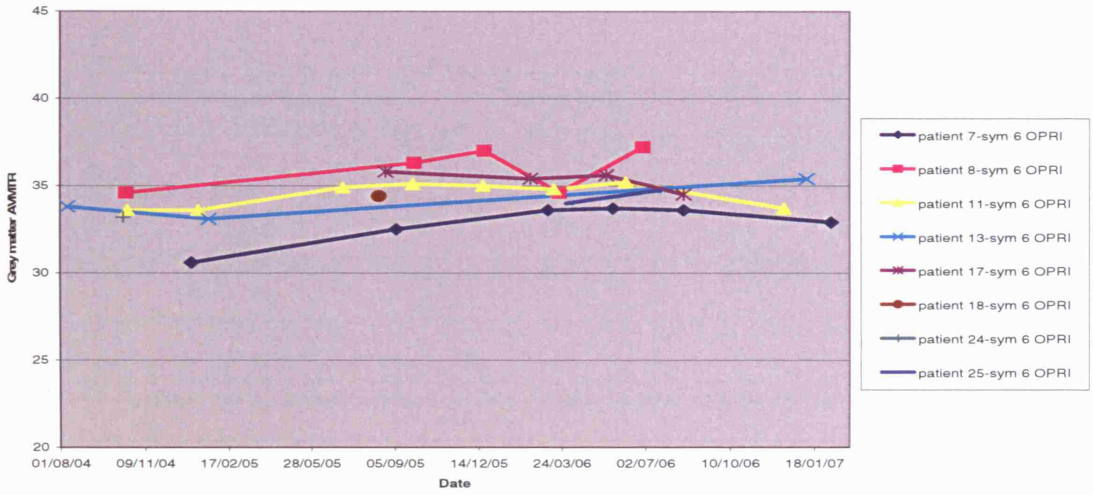
White matter AVMTR
01/08/04 09/11/04 17/02/05 28/05/05 05/09/05 14/12/05 24/03/06 02/07/06 10/10/06 18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

c)



d)

e)

Figure 34: a) MMSE in inherited symptomatic patients with 6 OPRI mutation. Only baseline MMSE was available for patients 8, 18, and 24, being 11, 9 and 21 respectively. MMSE for patients 7, 11, 13, 17 declined over time but remained stable at 28 for a total follow-up for 4 months for patient 25. b, c, d, e) Whole brain, white matter and grey matter AVMTR, and R caudate mean MTR in patients with 6 OPRI mutation either remains steady from the beginning, or reaches a plateau after an initial rise. In some patients there is a gradual return to baseline. Only in patient 8 is there an increase in all MTR measures over time, with longitudinal MMSE unavailable here. Longitudinal changes in remaining MTR ROI and histogram measures are described in Appendix R.

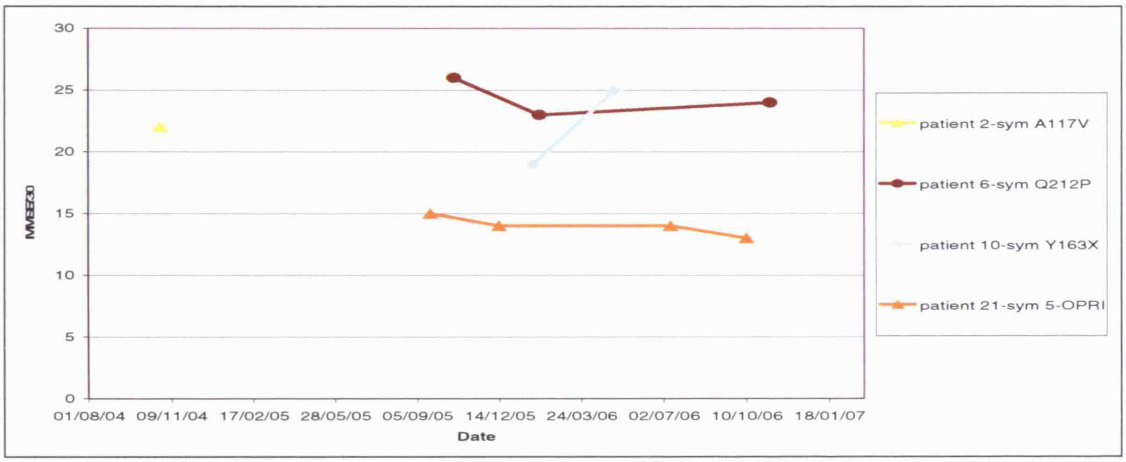


Figure 35: MMSE in inherited symptomatic patients with A117V, Q212P, Y163X and 5 OPRI mutations. Only baseline MMSE (22) was available for patient 2 with A117V mutation. MMSE declined from 26 to 24 in patient 6 with Q212P mutation, and from 15 to 13 in patient 21 with 5 OPRI mutation. MMSE increased from 21 to 25 in patient 10 with Y163X mutation, but follow up was only over 4 months

3.3.1.2 ADAS-COG

The Alzheimer's Disease Assessment Scale (ADAS-COG) is a 75 point scoring system that tests 12 areas: Word recall, Ideational praxis, Spoken language, Naming objects and fingers, Orientation, Word-finding difficulty in spontaneous speech, Commands, Word recognition, Comprehension, Constructional praxis, Remembering test instructions and Concentration/distractability. An increasing score on the ADAS-COG examination indicates worsening function.

A summary of ADAS-COG assessments in each trial participant is first shown. Patient 12 did not have ADAS-COG recorded at any stage because of expressive dysphasia, patient 17 either declined assessments (3) or SAP was used (1 assessment) where ADAS-COG was not recorded as MMSE was less than or equal to 10. Similarly, in patient 18 ADAS-COG was not recorded as MMSE was less than or equal to 10. All 3 patients had InhPrD (6 OPRI).

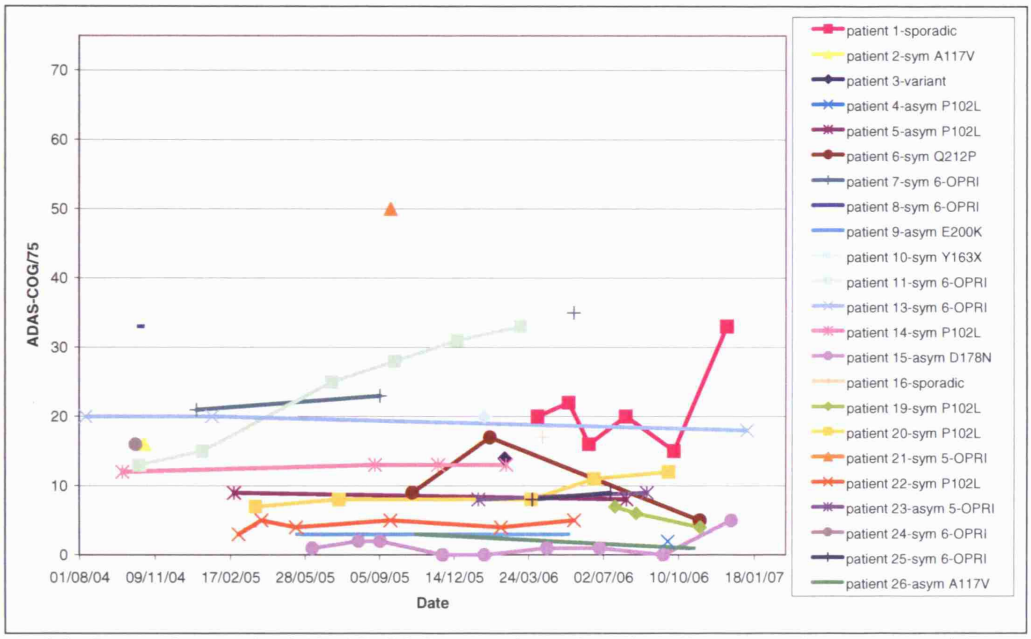


Figure 36: Composite graph of ADAS-COG scores in each patient. In most patients, scores either remain steady, or tend to increase, indicating clinical deterioration

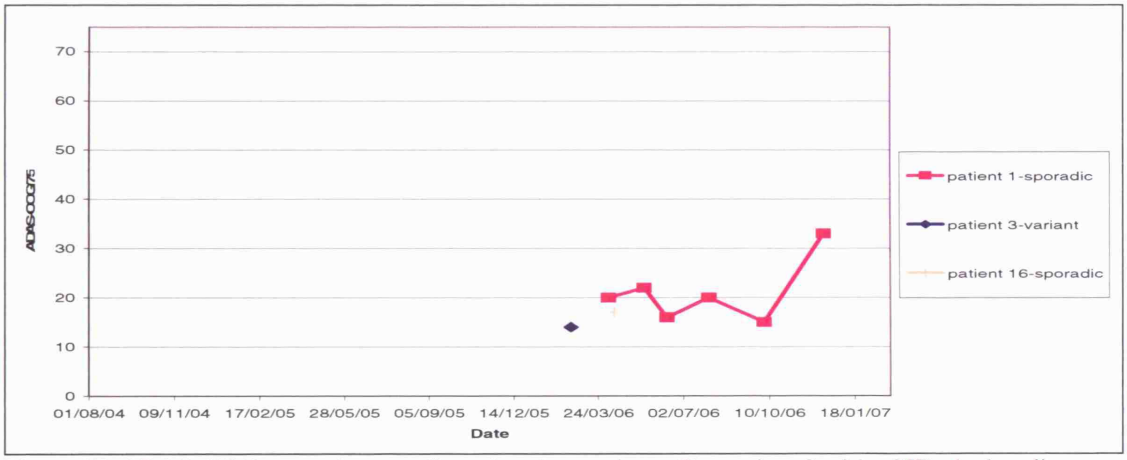


Figure 37: ADAS-COG scores in sporadic and variant patients. For patient 3 with sCJD, the baseline score was 20, which increased to 33 over a period of 8 months, indicating a rapid deterioration. The baseline score for patient 16 with sCJD was 17, and baseline score for patient 3 with vCJD was 14

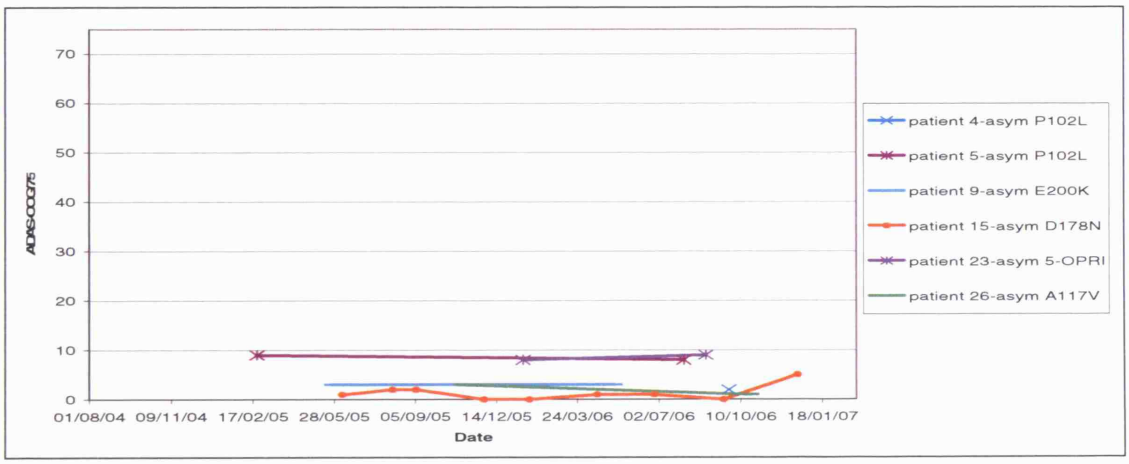


Figure 38: ADAS-COG scores in asymptomatic inherited patients. ADAS-COG scores are less than or equal to 9 in all asymptomatic patients, and do not change by more than 2 points, except in patient 15 with D178N mutation, where they increase from 1 to 5 over a period of 19 months, which may be a sign of early disease

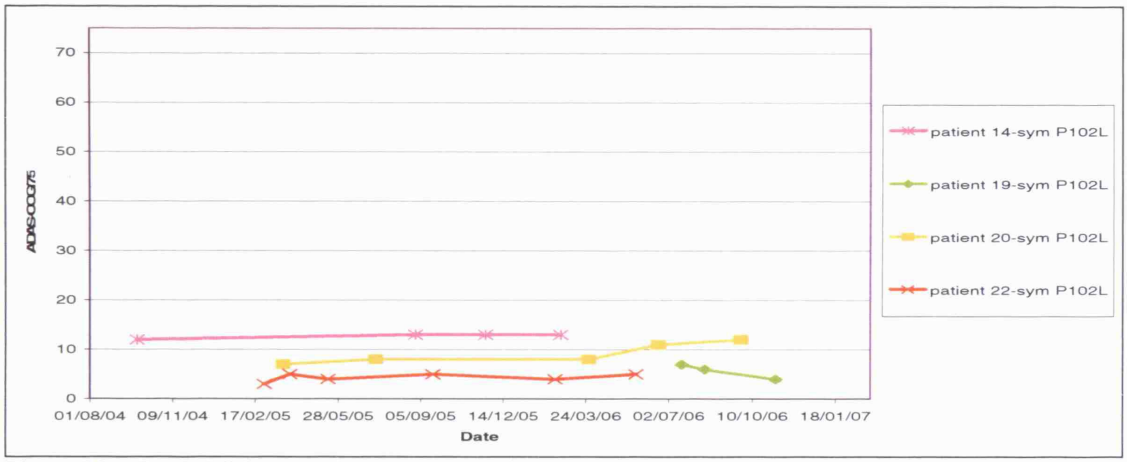


Figure 39: ADAS-COG scores in symptomatic inherited patients with P102L mutation. ADAS-COG scores have shown a gradual increase in all patients, except in patient 19, where it declined from 7 to 4, but the follow-up period was just four months

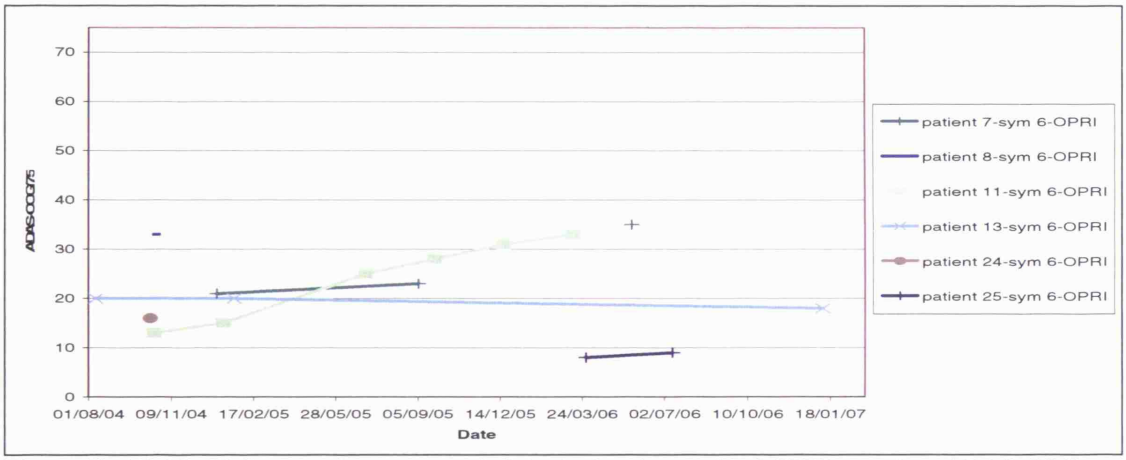


Figure 40: ADAS-COG scores in symptomatic inherited patients with 6 OPRI mutation. ADAS-COG scores have either shown a gradual increase (patient 11), or only shown a very minor change (patients 7, 13, 25). All patients have scores higher than 10 on baseline (patients 8, 24) or on longitudinal analysis (patients 7, 11, 13), except patient 25, with scores between 8-9, but only 2 assessments

3.3.1.3 Barthel ADL

The modified Barthel score used in the trial is a measure of activities of daily life (ADL), measuring 10 aspects of daily function (Bowels, Bladder, Grooming, Toilet use, Feeding, Transfers, Mobility, Dressing, Stairs and Bathing). The maximum

normal score is 20. Barthel scores decline with clinical deterioration and were recorded for all patients included in this thesis.

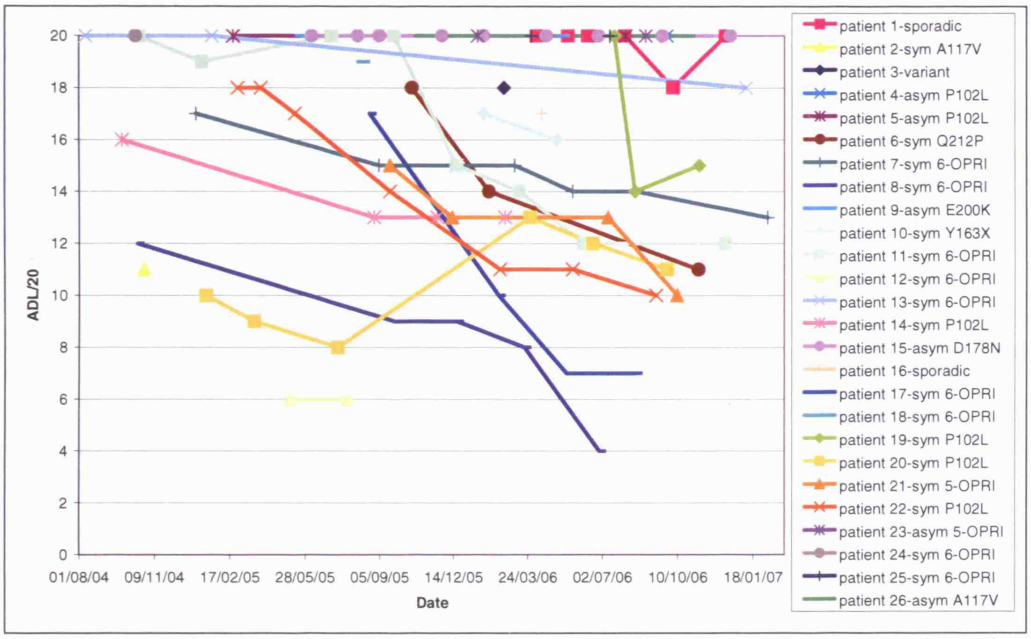


Figure 41: Composite graph of ADL scores in each patient. There is a general trend towards decline in most patients

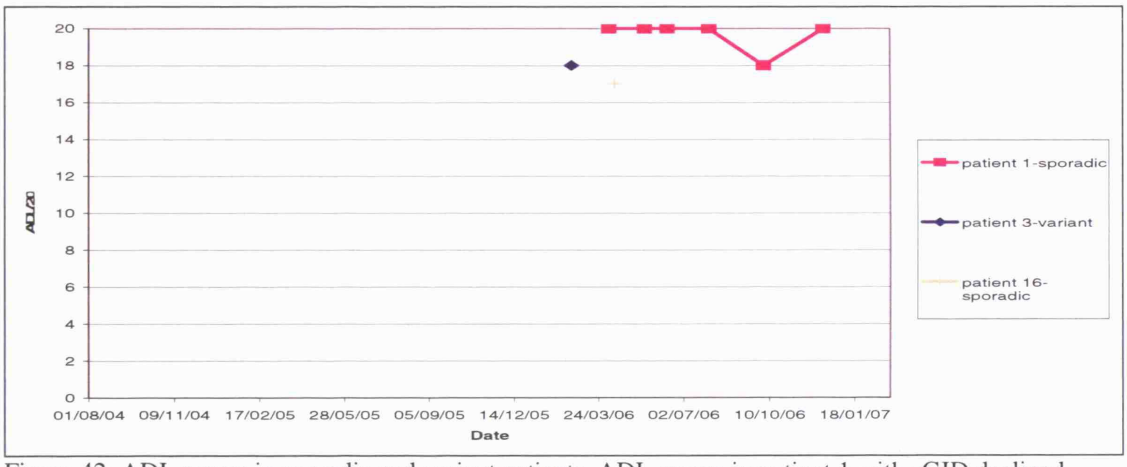


Figure 42: ADL scores in sporadic and variant patients. ADL scores in patient 1 with sCJD declined from 20 to 18, but returned to 20 over a follow-up period of 8 months

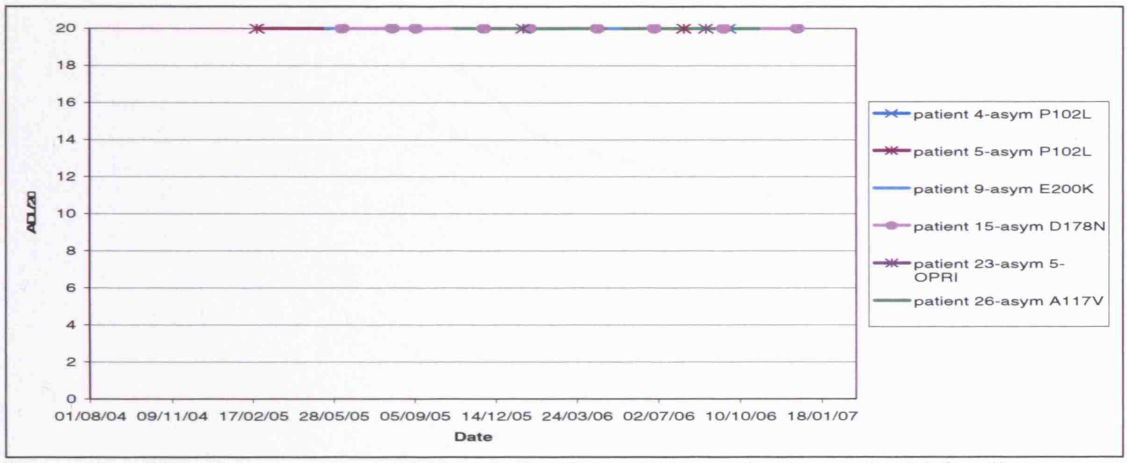
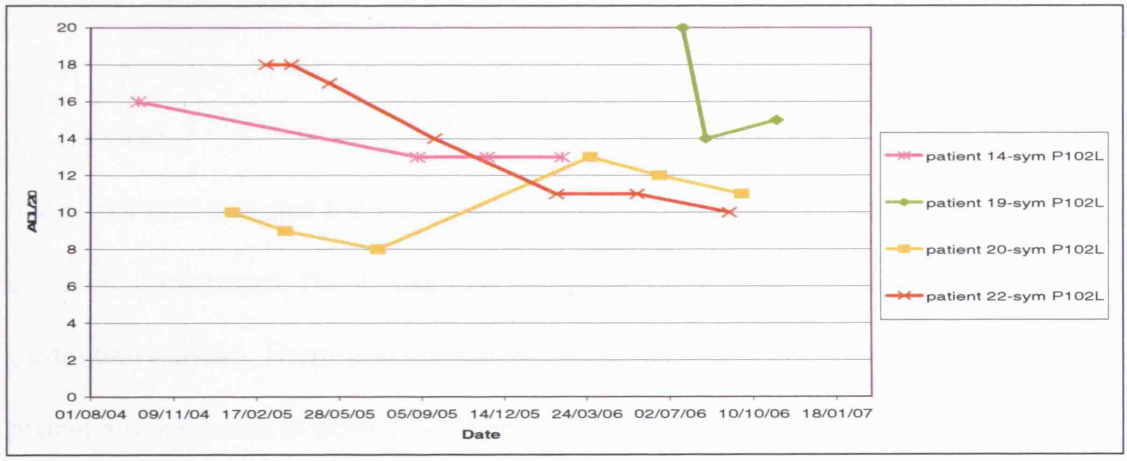
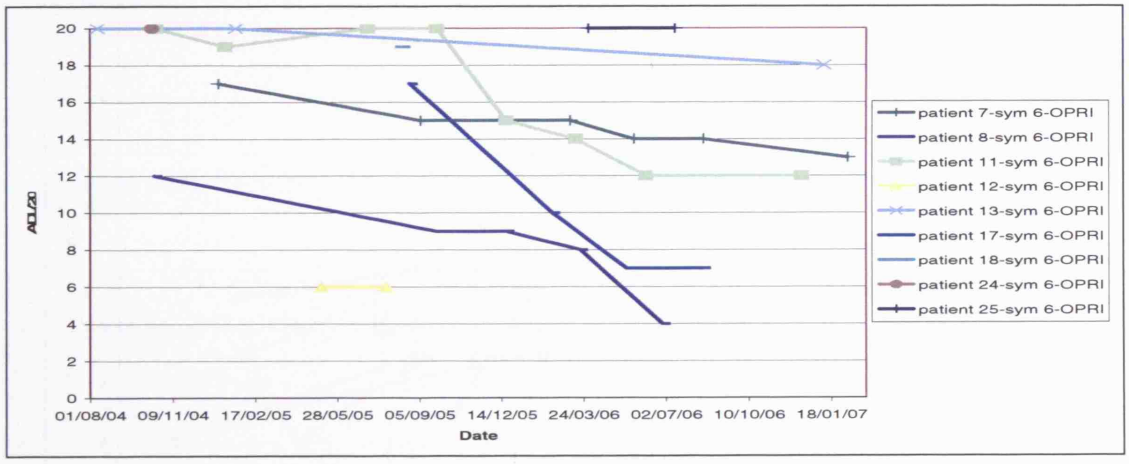


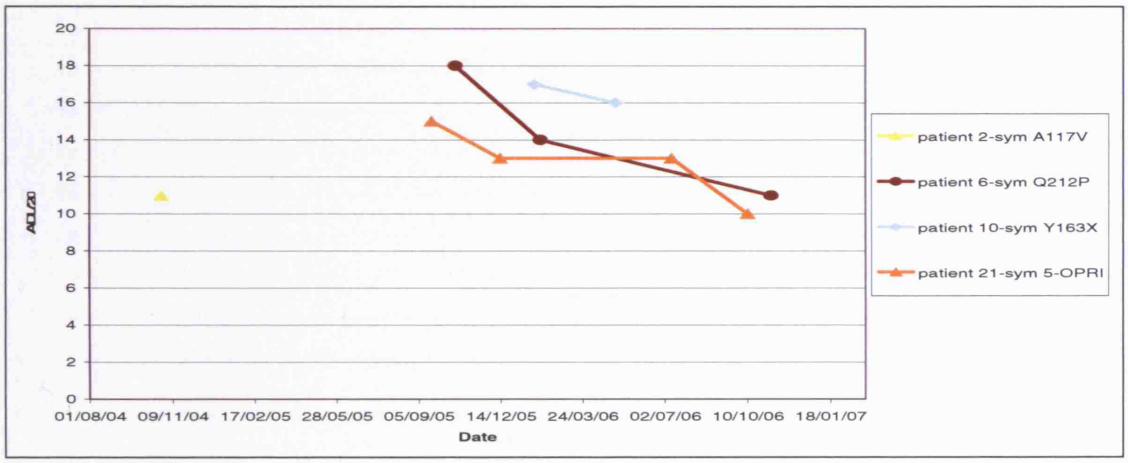
Figure 43: ADL scores in asymptomatic inherited patients. ADL remained normal at 20 for all asymptomatic inherited patients



a)



b)



c)

Figure 44: ADL scores in symptomatic inherited patients with a) P102L b) 6 OPRI c) Miscellaneous mutations. There is a general trend towards decline in ADL scores with time

3.3.1.4 CDR

Six domains of personal life are scored according to whether they are affected by cognitive impairment. The domains are: Memory, Orientation, Judgement, Community affairs, Home and hobbies and Personal care. CDR scores 0 in the normal patient and increases as dementia worsens, maximum score is 18. CDR increases with clinical deterioration and was recorded in all patients.

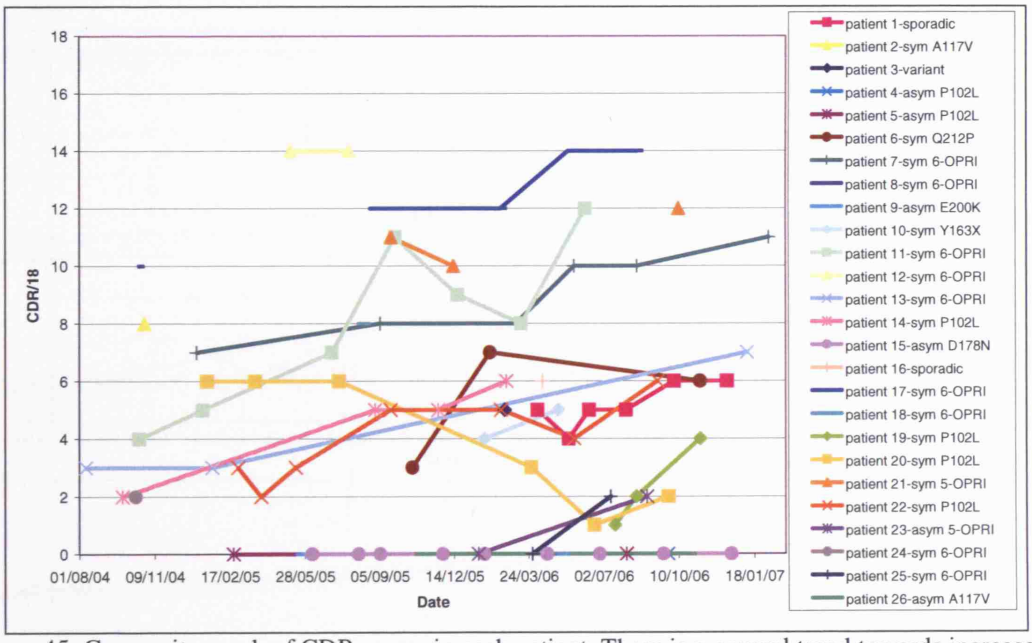


Figure 45: Composite graph of CDR scores in each patient. There is a general trend towards increase in clinical scores

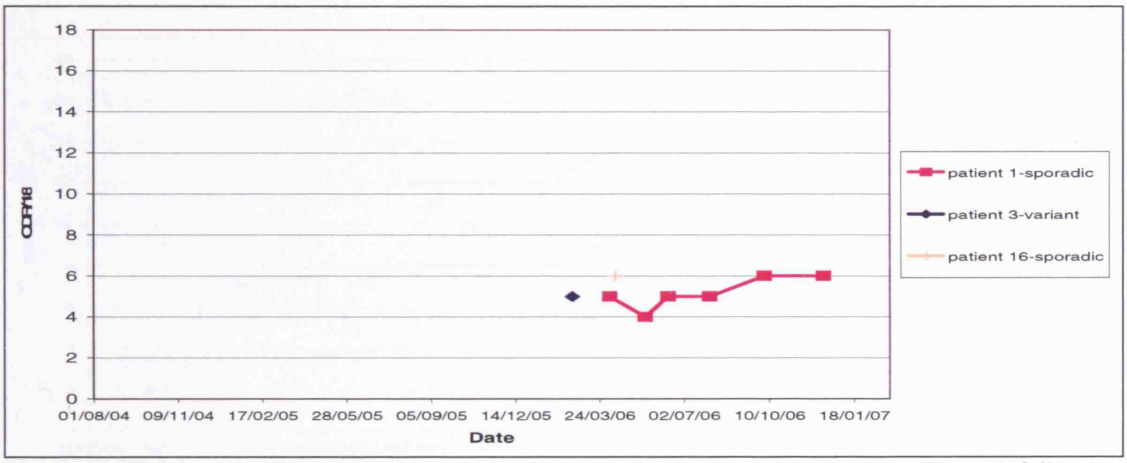


Figure 46: CDR in sporadic and variant patients. CDR remains between 4 to 6 over an 8 month follow-up period in patient 1 with sCJD. It was 5 and 6 at baseline in patient 3 with vCJD, and patient 16 with sCJD, respectively

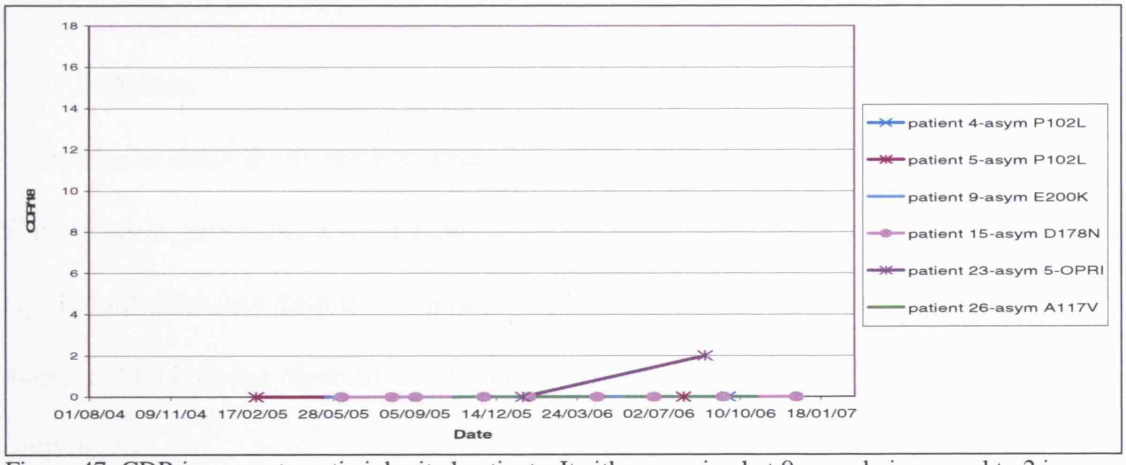
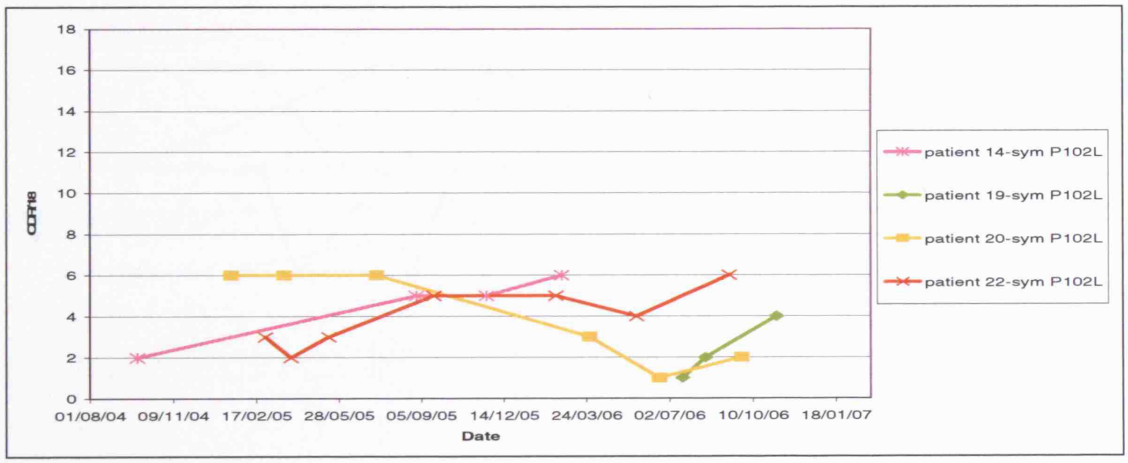
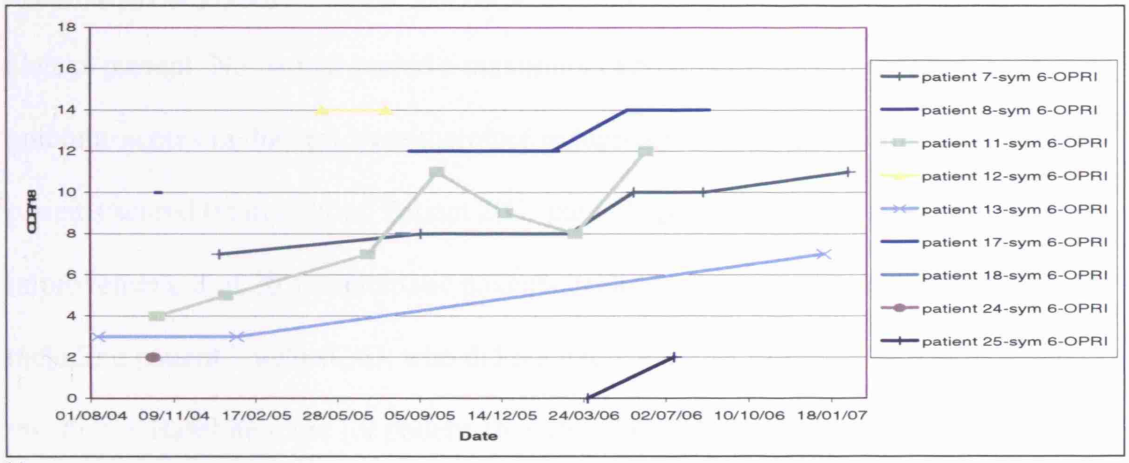


Figure 47: CDR in asymptomatic inherited patients. It either remained at 0, or only increased to 2 in patient 23 with 5 OPRI mutation



a)



b)

Figure 48: CDR scores in symptomatic inherited patients with a) P102L and b) 6 OPRI mutations. There is a general trend towards increase in CDR scores with time

3.3.1.5 Rankin

Activities of daily living were assessed in a more basic way than Barthel using the Rankin scale, providing a score ranging from 0 to 5 (0=no symptoms, 1=no significant disability despite symptoms, 2=slight disability, 3=moderate disability, 4=moderately severe disability, 5=severe disability). Unlike Barthel, scores increase with decline.

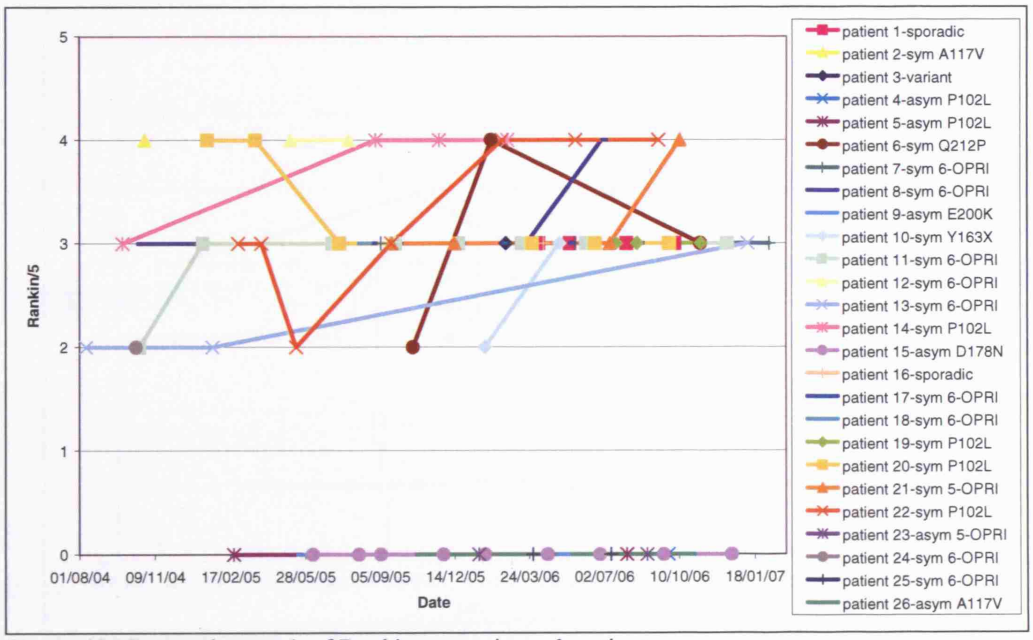


Figure 49: Composite graph of Rankin scores in each patient

There is a notable absence of scores of 1 in this test (no significant disability despite

symptoms); implying that once you have symptomatic prion disease, disability is always present. No patient scored a maximum of 5. An already limited number of outcome scores in this test were therefore reduced even more. All asymptomatic patients scored 0 throughout. Patient 20 is the only patient showing an overall improvement. 8 of 20 symptomatic patients declined, the rest had stable scores, including patient 1 with sCJD, who did not decline in the follow-up period included in this thesis. Baseline score for patient 16 with sCJD and patient 3 with vCJD was 3, indicating that these patients were at least moderately disable when assessed.

3.3.1.6 CGIS

A clinician's global impression of disease severity, as assessed by the doctor is shown here. Scores are 1-7, increasing with increasing severity: 1=Normal, not ill at all, 2= Borderline mentally ill, 3=Mildly ill, 4= Moderately ill, 5=Markedly ill, 6 Severely ill, 7=Amongst the most ill. This is the most subjective test of all discussed in this section.

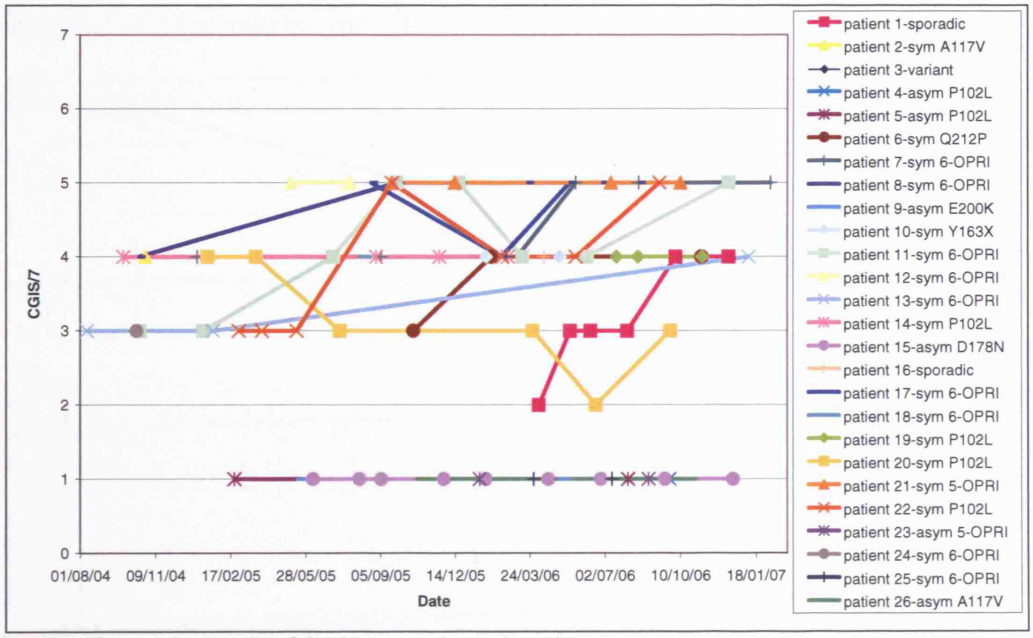


Figure 50: Composite graph of CGIS scores in each patient

Patterns are most similar to Rankin scores as the scoring structure is almost identical and on a similar seven point scale. Score 2 is little used as 'Borderline mentally ill' is perhaps too narrow a description for the multifaceted neurological degeneration seen in these patients. No patients achieved the worst possible scores of 6 or 7, because none of the patients included in this thesis were 'severely ill' or 'amongst the most ill'. All asymptomatic patients scored 1. Like the Rankin score this is a simple test, which was recorded in all patients, but outcome scores are closely bunched. Similarly, patient 20 is the only patient showing an overall improvement. Baseline scores for both patient 16 with sCJD and patient 3 with vCJD was 4, indicating that these patients were moderately ill when assessed, however patient 1 with sCJD scored 2 (borderline mentally ill) at baseline, but their scores rose sharply to 4 (moderately ill) over a period of 8 months.

3.3.1.7 GCS

GCS is a measure of level of consciousness, scored between 15 (maximum) and 3 (minimum). All patients in whom GCS was recorded had a GCS of 10 or more.

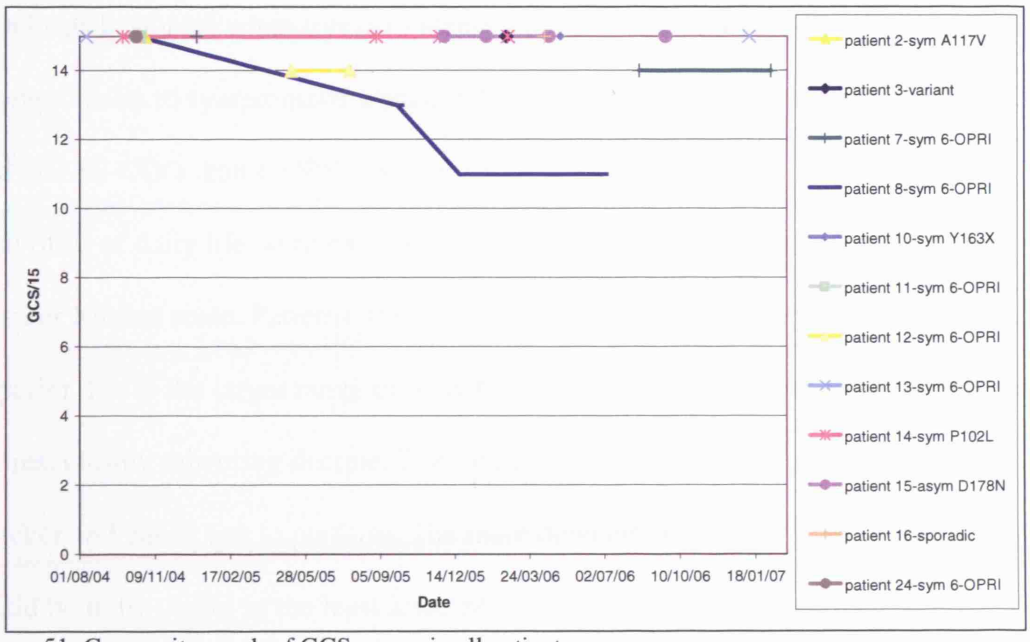


Figure 51: Composite graph of GCS scores in all patients

GCS was recorded in 12/26 patients. In patients 2, 3, 10, 11, 16 and 24, with sporadic, inherited and variant prion disease, it was either recorded at baseline or follow-up as a single value of 15. In patients 7, 8, 12, 13, 14 and 15, with inherited prion disease, follow-up values were recorded, ranging from 11-15, though values from some assessments were missing. In patient 8 with symptomatic inherited prion disease due to 6 OPRI, where GCS was recorded at all follow-up visits over a period of 9 months, it declined from 15 to 11. Thus, it may be a useful clinical measure in prion disease for demonstrating decline, when appropriately recorded.

3.3.1.8 Summary of findings in non-videoed neurological assessments

The non-videoed assessments such as MMSE, ADAS-COG, Rankin, Barthel, CDR and CGIS all produced similar profiles of results in the disease and severity groups described.

In the complex cognitive tests MMSE had the advantage of a more complete data set than ADAS-COG, as it is easier to perform. Although scores fluctuated in patients, MMSE captured useful information on a larger subset of trial patients. The ADAS-COG was best used when trying to identify the point at which a patient changes from asymptomatic to symptomatic status. P102L patients scored better on both MMSE and ADAS-COG than 6 OPRI patients.

Activities of daily life were measured with the more complex Barthel ADL and simpler Rankin scale. Patterns of decline were the same in both tests but ADL was superior due to the larger range of activities considered and greater range of outcome scores, closely mirroring decline. The range of scores in Rankin was low but it is a quicker and easier test to perform. The more detailed assessment used in Barthel ADL could be more useful in the least affected or asymptomatic patients. ADL scores in 6

OPRI patients declined once the SAP was required, demonstrating global deficits in these patients.

CDR scores different functions to the tests of activities of daily life discussed above and provides a useful comparison. There was a general trend towards increase in CDR scores in symptomatic patients.

CGIS is the most subjective of the tests as it is dependent on the rater's opinion which may be affected by knowledge of disease type and length of symptoms. However, it is also free from restraints of fixed determined outcome scores that other tests have. Results mirrored those of the Rankin score, possibly due to the similar scoring system.

GCS is a very useful tool in assessment of severely affected patients and across all CJD types. Its utility was compromised by a high rate of missing values in this study. This test should certainly be performed and due to its simplicity could be reliably completed in all patients at all stages of disease (an uncommon virtue amongst tests).

3.3.2 Longitudinal and baseline assessment of videoed clinical scores

Scores for cognitive, extrapyramidal, pyramidal and cerebellar impairment were analysed by an independent neurologist on a 4-point scale (0 (none), 1 (mild), 2 (moderate), 3 (severe), 4 (cannot assess)). BPRS scores were not independently analysed.

3.3.2.1 Cognitive impairment

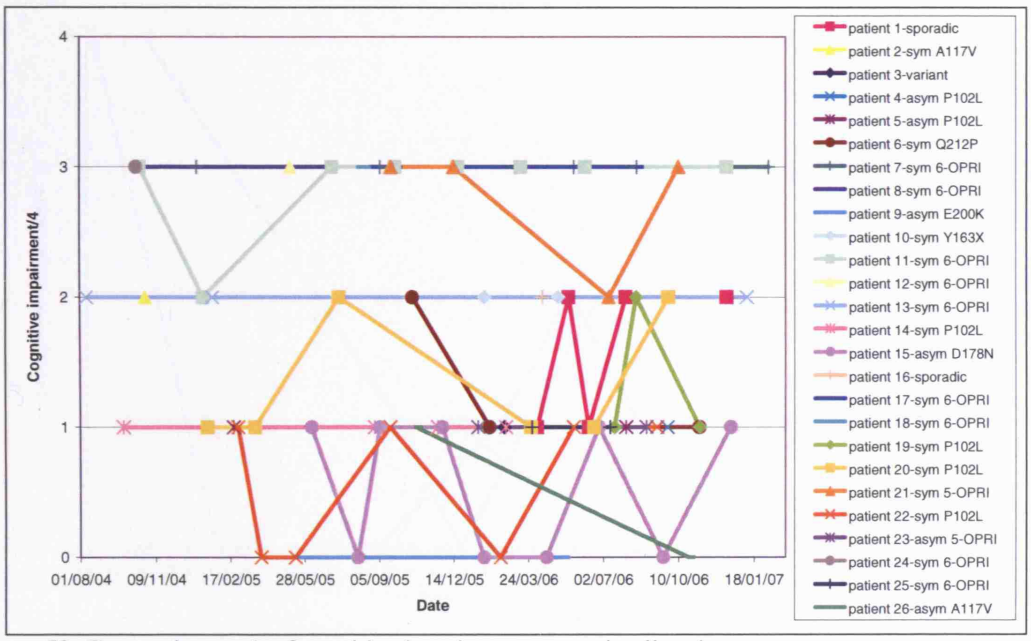


Figure 52: Composite graph of cognitive impairment scores in all patients

Even though the patients tend to fluctuate, a score of 0 (no impairment) is only attained by the asymptomatic patients or by patient 22 with P102L mutation, whose MMSE has remained 29-30 throughout 18 months of follow-up who predominantly has ataxia, the commonest mode of presentation for this mutation. All the remaining symptomatic patients, including inherited, sporadic and variant patients have remained mildly (1) to severely (3) impaired on baseline and longitudinal analysis. Independent video analysis of cognitive impairment was a useful tool in prion disease as it could be assessed in all patients and was a robust measure of change in cognition.

3.3.2.2 Extrapyramidal impairment

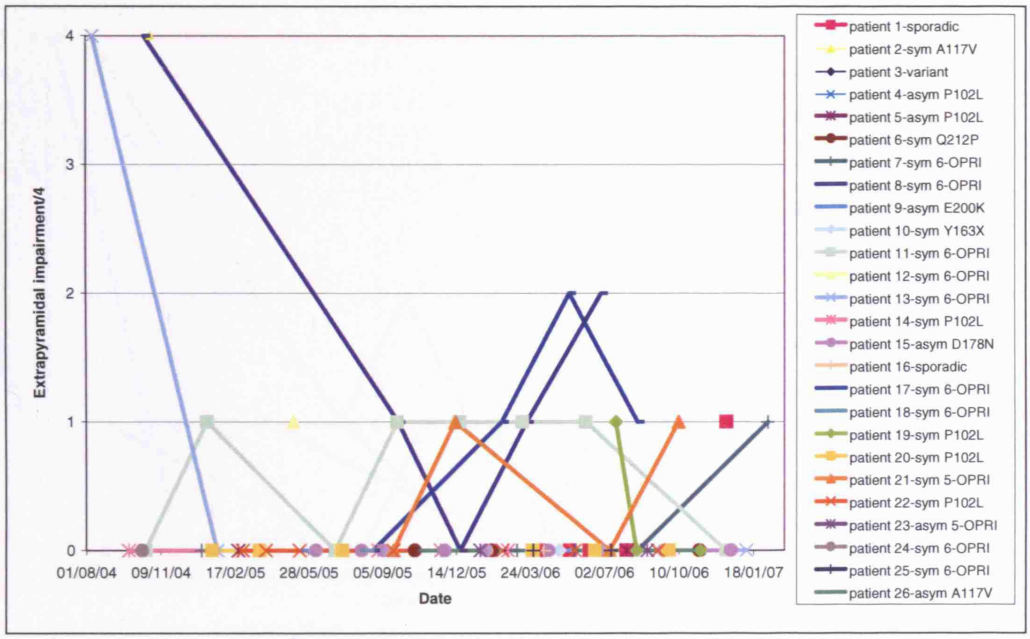


Figure 53: Composite graph of extrapyramidal impairment scores in all patients

There was no extrapyramidal impairment (0) in asymptomatic inherited, sporadic, variant and symptomatic inherited patients with P102L mutation, except in patient 19 with P102L mutation, who had mild impairment (1). None of the patients had severe impairment (3). Moderate impairment was only seen in patients with 6 OPRI mutation. Extrapyramidal impairment could not be assessed in three patients with inherited prion disease (2 6 OPRI, 1 A117V) at baseline. It is a motor score which can sometimes be difficult to assess on an independent video assessment.

3.3.2.3 Pyramidal impairment

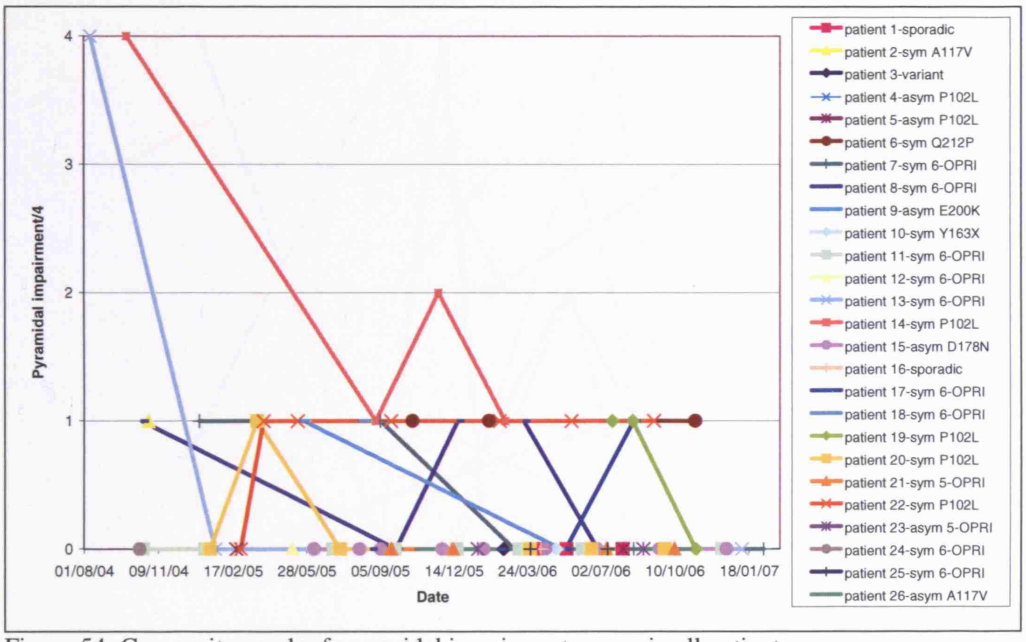


Figure 54: Composite graph of pyramidal impairment scores in all patients

There was no pyramidal impairment (0) in asymptomatic inherited, sporadic and variant patients, except mild impairment (1) in patient 9 asymptomatic with E200K mutation. It could not be assessed in two patients symptomatic with InhPrD. This suggests that pyramidal impairment may also be difficult to assess on an independent video assessment.

None of the patients had severe impairment (3). Mild (1) to moderate (2) impairment was only seen in patients symptomatic with inherited prion disease.

3.3.2.4 Cerebellar impairment

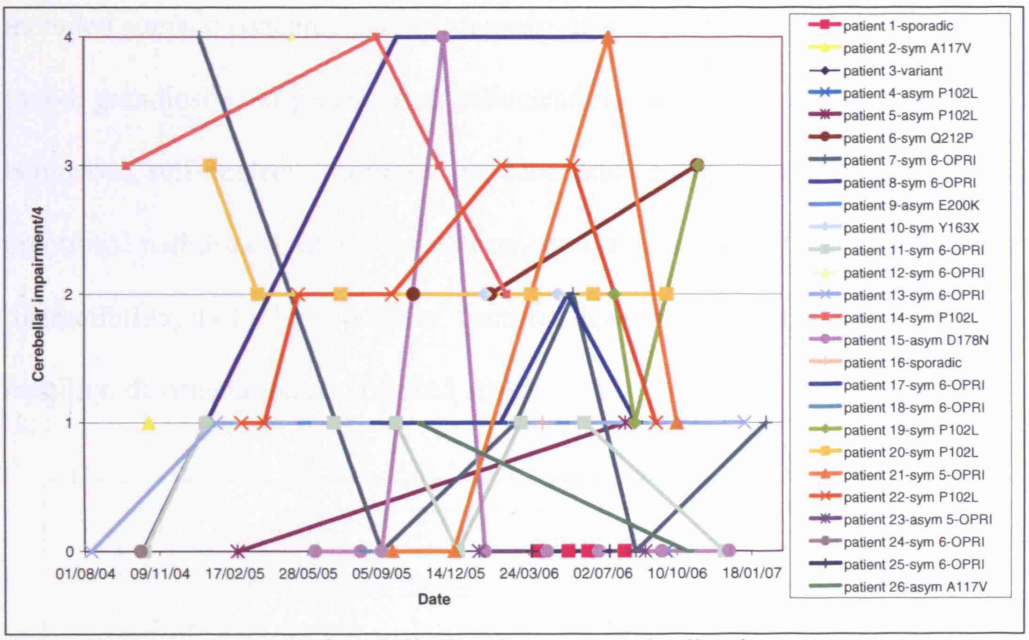


Figure 55: Composite graph of cerebellar impairment scores in all patients

This graph shows fluctuations, particularly because cerebellar impairment could not be assessed at baseline or longitudinal analysis in different patients, either because the patients were unable to walk to demonstrate gait ataxia or it was difficult to assess their gait on video. The video examination is open to interpretation in this case as well, especially as mild impairment (1) was thought to be the case in 3 patients asymptomatic with InhPrD and no assessment (4) could be made in another asymptomatic InhPrD patient.

Severe impairment (3) was only seen in patients with P102L mutation while the remainder of symptomatic InhPrD, sporadic and variant patients showed none (0), mild (1) or moderate (2) impairment at baseline or longitudinal analysis.

3.3.2.5 BPRS

The BPRS scored 24 items on a qualitative scale from 1 to 7 (according to whether the symptom was absent, very mild, mild, moderate, moderately severe, severe or extremely severe) providing a final score ranging from 24 to 168. Components

included somatic concern, anxiety, depression, suicidality, guilt, hostility, elated mood, grandiosity, suspiciousness, hallucinations, unusual thought content, bizarre behaviour, self-neglect, disorientation, conceptual disorganisation, blunted affect, emotional withdrawal, motor retardation, tension, uncooperativeness, excitement distractibility, motor hyperactivity, mannerisms and posturing, anxiety, depression, hostility, disorientation and blunted affect.

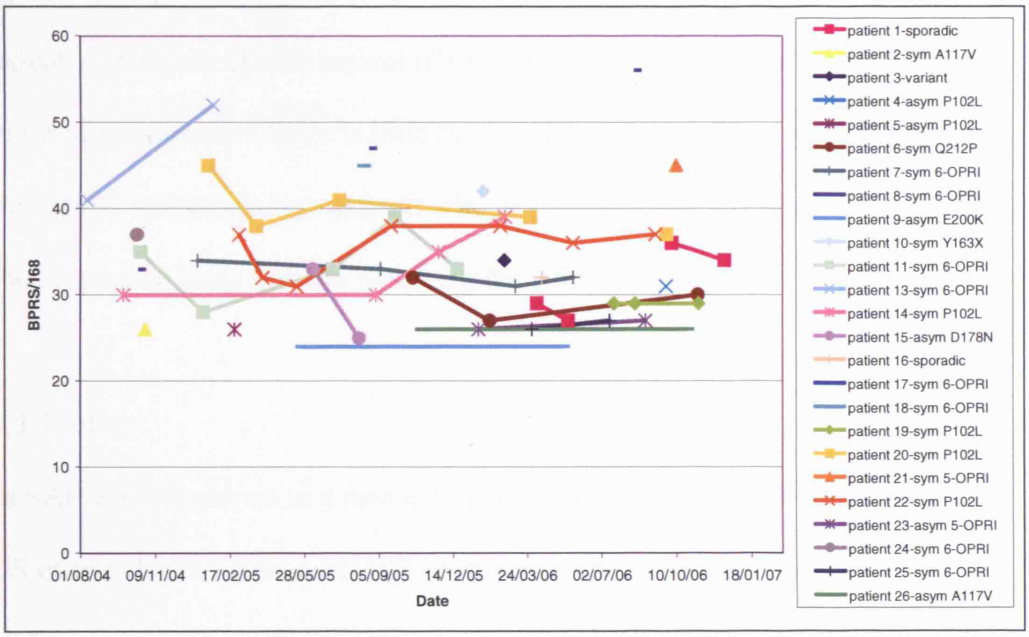


Figure 56: Composite graph of BPRS scores in all patients. BPRS scores have remained steady on the whole without any significant rise or decline

3.3.2.6 Summary of findings in videoed neurological assessments

The 2 sporadic patients show very different patterns of impairment at baseline, 1 with mild cognitive and no extrapyramidal impairment (Patient 1, progressing to develop only moderate cognitive impairment longitudinally) and the second (Patient 16) with moderate cognitive and mild extrapyramidal impairment. The variant patient (Patient 3) had mild cognitive and moderate cerebellar impairment at baseline. The most striking result from the asymptomatic group is how many measures are deemed as impaired; mild cognitive, cerebellar and pyramidal impairment were seen.

In the symptomatic inherited patients the 6 OPRI group had moderate or severe cognitive impairment and less severe cerebellar impairment. The P102L group had moderate or severe cerebellar impairment and less severe cognitive impairment. Pyramidal and extrapyramidal function was normal or at most mildly impaired in all patients.

Other inherited patients, such as the 5 OPRI patient (Patient 21), were severely cognitively impaired at baseline and on longitudinal analysis. The Y163X patient (Patient 10) and the Q212P patient (Patient 6) had a mixed cognitive and cerebellar pattern of impairment. There is little firm evidence for pre-terminal decline on longitudinal analysis in two patients who have passed away (Patients 14 and 17). BPRS remained steady on the whole for the whole group.

3.3.2.7 SAP

The SAP was developed as a means of assessing patients in the PRION-1 trial with a GCS of less than 15 and an MMSE of less than 10. These patients were usually unable to perform any videoed cognitive tests and few motor tests, thus a subset of easier tests was selected for this cohort. Five 6 OPRI patients required the SAP during the trial. There was a large group of patients in the PRION-1 trial who were assessed using the SAP but were not included in this analysis as they did not have MRI scans. Analysis of the use of SAP in the patients discussed here are therefore not necessarily representative of the group requiring the SAP as a whole. The group described here are the 'high performers' who were either initially well enough to have an MRI scan or remained well enough to continue having MRI scans. In general, those not discussed here started at the lowest scores in the SAP and further decline could not be measured due to the flooring effect of the scale.

One 6 OPRI inherited patient (Patient 18) had SAP at baseline. She was severely cognitively impaired. The remaining 4 6 OPRI patients (Patients 18, 8, 7 and 11) were followed up over a minimum time interval of 2 months and a maximum time interval of 109 months, having 2/2, 4/5, 2/6 and 2/8 assessments as SAP respectively. Though all 4 patients were severely cognitively impaired, individual memory scores were mostly not recordable, eye movements could not be assessed due to lack of following and the finger nose, rapid alternating movements, sequential index finger tapping and opposition could not be scored. Primitive reflexes were present in all assessments and there were no abnormal movements on observation. Neurological examination was abnormal either in tone, power or reflexes in all patients.

The SAP was thus a useful indicator of patients who had declined cognitively to the point where simple tests of cognition could not be attempted or completed. Beyond this there was a mixture of abilities, but a large amount of flooring effects, even in this 'highly performing' group.

A potential source of bias in using the SAP was that tests previously used in the full video but not used in the SAP showed missing values. In general, it was the cognitive tests that were removed in the SAP and although an independent analysis could not be made from individual tests (for example spelling, calculation etc.) an overall assessment of cognitive state of the patient was made from their clinical condition as seen on video by the independent neurologist. No cognitive tests tended to show conservation of function when other tests had declined. If the SAP had not been used the individual cognitive test results would still have been missing as the patient would have been unable to perform them.

3.4 FLAIR/DWI ANALYSIS

This is summarised in Table 7.

Table 7: Summary of DWI/FLAIR findings on baseline and longitudinal analysis

No.	Type	DWI		FLAIR	
		Signal abnormality		Signal abnormality	
		No	Yes	No	Yes
1	sCJD		Yes (Hyperintensity in frontal, temporal, occipital and cingulate cortices bilaterally)		Yes (Hyperintensity in caudate, pulvinar, frontal, temporal, occipital and cingulate cortices bilaterally)
2	A117V	No		No	
3	vCJD		Yes (Hyperintensity in pulvinar bilaterally)		Yes (Hyperintensity in pulvinar bilaterally)
4	P102L	No		No	
5	P102L	No		No	
6	Q212P	No		No	
7	6 OPRI	No		No	
8	6 OPRI	No		No	
9	E200K	No		No	
10	Y163X	No		No	
11	6 OPRI	No		No	
12	6 OPRI	No		No	
13	6 OPRI	No		No	
14	P102L	No		No	
15	D178N	No		No	
16	sCJD		Yes (hyperintensity in parietal, temporal, occipital & cingulate cortices bilaterally)		Yes (hyperintensity in parietal, temporal, occipital & cingulate cortices bilaterally)
17	6 OPRI		Yes (hyperintensity in frontal, parietal & cingulate cortices bilaterally)		Yes (hyperintensity in frontal, parietal & cingulate cortices bilaterally)

18	6 OPRI	No		No	
19	P102L	No		No	
20	P102L	No		No	
21	5 OPRI	No		No	
22	P102L	No		No	
23	5 OPRI	No		No	
24	6 OPRI	No		No	
25	6 OPRI	No		No	
26	A117V	No		No	

At baseline, patients 1 and 16 with sporadic prion disease, patient 3 with variant prion disease and patient 17 with inherited prion disease had qualitative DWI/FLAIR changes (Figures 57 a, b and c). No longitudinal data was available for patients 3 (vCJD) and16 (sCJD) but baseline changes persisted on longitudinal analysis in patients 1 (sCJD) and 17 (InhPrD).

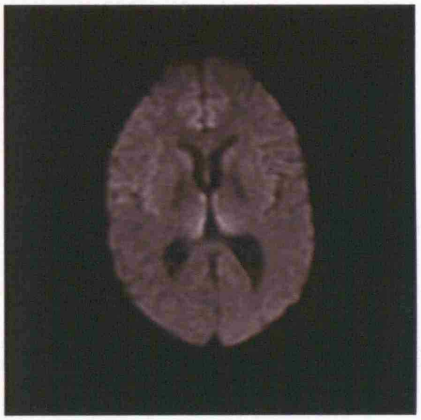
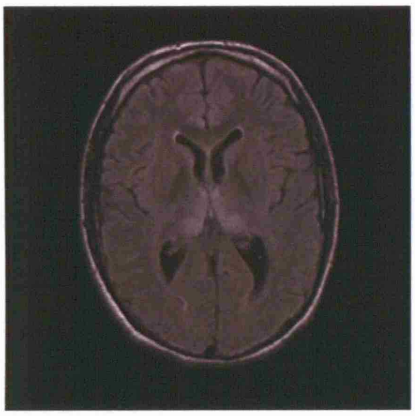
1)  2) 

Figure 57a: 1) DWI and 2) FLAIR sequences in patient 3 with vCJD

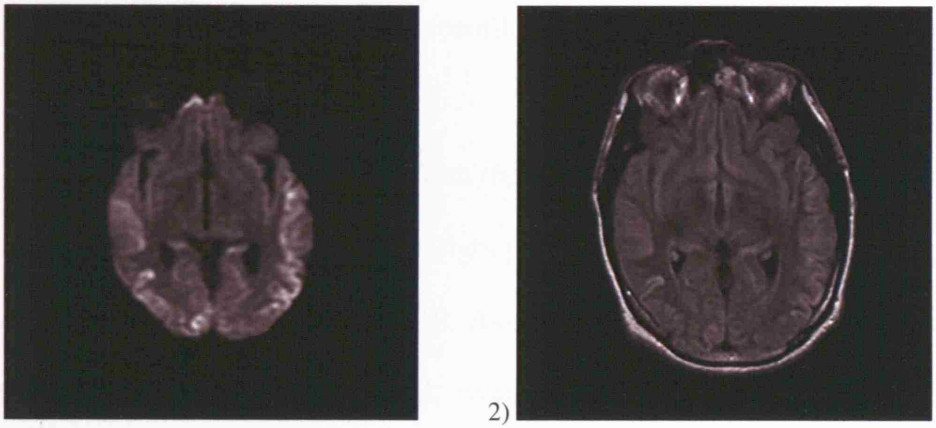


Figure 57b: 1) DWI and 2) FLAIR sequences in patient 16 with sCJD

Figure 57c: 1) DWI and 2) FLAIR sequences in patient 17 with inherited prion disease

3.5 RESULTS OF STATISTICAL ANALYSIS

3.5.1 Correlation between MT measures and clinical scores at baseline in all patients

This section describes statistically significant correlations between MT measures and clinical scores at baseline in all patients. No such correlations could be established when analysis was carried out separately for the smaller symptomatic (n=20) and asymptomatic (n=6) patient groups. A summary of MT and clinical measures at baseline, used to establish these correlations, is given in Appendix S. Spearman rank correlation analysis was performed to correlate MT measures derived from:

a) Whole brain, white matter and grey matter histogram analyses (Average MTR (AVMTR), Peak Height (PH), Peak Location (PL), MTR at 25th percentile

(MTR25%), MTR at 50th percentile (MTR50%), and MTR at 75th percentile (MTR75%)), and

b) ROI analysis (Mean MTR from right caudate nucleus, left caudate nucleus, right putamen, left putamen, right pulvinar and left pulvinar)

with clinical measures (MMSE, CDR, ADAS-COG, Rankin, BPRS, CGIS, ADL, GCS, and cognitive, extrapyramidal, pyramidal and cerebellar impairment) in both symptomatic and asymptomatic patients. On independent sample t-test, whole brain AVMTR ($p=0.007$) and MTR50% ($p=0.05$), white matter AVMTR ($p=0.01$), MTR25% ($p=0.03$) and MTR75% ($p=0.007$), and grey matter AVMTR ($p=0.004$), MTR25% ($p=0.005$) and MTR50% ($p=0.008$) were significantly ($p<0.05$) different between symptomatic ($n=20$) and asymptomatic ($n=6$) patients.

Cortical hyperintensity on DWI/FLAIR was observed in one patient symptomatic with InhPrD and DWI/FLAIR hyperintensities in cortex, basal ganglia and pulvinar regions were observed in two sCJD and one vCJD patient, all were symptomatic with the disease. The remaining patients did not have any abnormalities on these conventional sequences at baseline. There was no significant ($p<0.05$) difference in mean ROI MTRs or whole brain, white matter and grey matter MTR histogram measures derived from patients with normal ($n=20$) and abnormal ($n=4$) conventional imaging when assessed using an independent sample t-test.

The above correlation involved multiple testing between twenty-four MTR outcomes and twelve clinical outcomes, thus a total of $24\times12=288$ tests were carried out. If it was assumed that all these measures were independent and a Bonferroni correction applied to a normal p value of 0.05, then a p value of 0.000174 ($0.05/ (24\times12)$) would have been considered significant at the 0.05 level allowing for multiple testing. However, clinical rating scales are not likely to be entirely independent. Consistent

associations between the various clinical rating scales and different MTR measures was noted and $p<0.01$ was used to identify potentially significant associations in the small number of patients in this study. This method does increase the possibility for false positives.

Expected associations would be that a decline in MT measures was associated with a decline in a patient's clinical condition, indicated by:

a) Worsening MMSE as scores decreased from 30 to 0
b) Worsening ADL as scores decreased from 20 to 0
c) Worsening ADAS-COG as scores **increased** from 0 to 75
d) Worsening CDR as scores **increased** from 0 to 18
e) Worsening CGIS as scores **increased** from 1 to 7
f) Worsening GCS as scores decreased from 15 to 3
g) Worsening Rankin as scores **increased** from 0 to 5
h) Worsening BPRS as scores **increased** from 24 to 168
i) Worsening cognitive impairment as scores **increased** from 0 (no impairment) to 4 (not assessed)
j) Worsening extrapyramidal impairment as scores **increased** from 0 (no impairment) to 4 (not assessed)
k) Worsening pyramidal impairment as scores **increased** from 0 (no impairment) to 4 (not assessed)
l) Worsening cerebellar impairment as scores **increased** from 0 (no impairment) to 4 (not assessed)

Significant correlations ($p<0.01$) in this analysis have been highlighted in yellow in Tables 8, 9 and 10, with their graphic representation in Figure 58.

Table 8: Correlations between MT measures and MMSE, ADL, ADAS-COG and CDR at baseline in all patients

MT measures		MMSE	ADL	ADAS-COG	CDR
a) Whole brain histogram analysis					
AVMTR	Correlation Coefficient	0.47	0.27	-0.54	-0.53
	p value	0.02	0.18	0.01	0.005
PH	Correlation Coefficient	0.11	0.16	-0.17	-0.43
	p value	0.59	0.45	0.45	0.03
PL	Correlation Coefficient	0.02	-0.06	-0.18	-0.03
	p value	0.93	0.78	0.42	0.88
MTR25%	Correlation Coefficient	0.51	0.34	-0.51	-0.62
	p value	0.009	0.09	0.02	0.001
MTR50%	Correlation Coefficient	0.25	0.19	-0.38	-0.38
	p value	0.24	0.37	0.08	0.05
MTR75%	Correlation Coefficient	0.20	0.04	-0.40	-0.17
	p value	0.33	0.86	0.07	0.38
b) White matter histogram analysis					
AVMTR	Correlation Coefficient	0.18	0.23	-0.30	-0.33
	p value	0.39	0.25	0.17	0.10
PH	Correlation Coefficient	-0.09	0.03	0.06	-0.16
	p value	0.68	0.87	0.80	0.43
PL	Correlation Coefficient	0.24	0.20	-0.34	-0.33
	p value	0.25	0.32	0.12	0.09
MTR25%	Correlation Coefficient	0.18	0.16	-0.24	-0.30
	p value	0.40	0.43	0.29	0.14
MTR50%	Correlation Coefficient	0.18	0.28	-0.31	-0.33
	p value	0.39	0.17	0.16	0.10
MTR75%	Correlation Coefficient	0.32	0.14	-0.45	-0.30
	p value	0.12	0.50	0.03	0.13
c) Grey matter histogram analysis					
AVMTR	Correlation Coefficient	0.39	0.25	-0.50	-0.47
	p value	0.05	0.23	0.02	0.02
PH	Correlation Coefficient	0.47	0.18	-0.37	-0.56
	p value	0.02	0.37	0.09	0.003
PL	Correlation Coefficient	0.21	0.18	-0.30	-0.33
	p value	0.32	0.38	0.17	0.10
MTR25%	Correlation Coefficient	0.38	0.19	-0.42	-0.47
	p value	0.06	0.36	0.05	0.02
MTR50%	Correlation Coefficient	0.40	0.16	-0.51	-0.44
	p value	0.05	0.44	0.02	0.03
MTR75%	Correlation Coefficient	0.30	0.22	-0.48	-0.31
	p value	0.15	0.29	0.02	0.12

d) ROI analysis					
Right caudate	Correlation Coefficient	0.26	0.37	-0.18	-0.28
	p value	0.21	0.06	0.43	0.16
Left caudate	Correlation Coefficient	0.43	0.32	-0.48	-0.42
	p value	0.03	0.11	0.03	0.03
Right putamen	Correlation Coefficient	0.08	0.18	-0.22	-0.11
	p value	0.69	0.38	0.33	0.61
Left putamen	Correlation Coefficient	0.21	0.31	-0.28	-0.29
	p value	0.33	0.13	0.20	0.16
Right pulvinar	Correlation Coefficient	0.10	0.02	-0.21	-0.06
	p value	0.648	0.94	0.36	0.75
Left pulvinar	Correlation Coefficient	0.05	0.16	-0.28	-0.18
	p value	0.81	0.44	0.20	0.38

Table 9: Correlations between MT measures and CGIS, GCS, Rankin and BPRS at baseline in all patients

MT measures		CGIS	GCS	Rankin	BPRS
a) Whole brain histogram analysis					
AVMTR	Correlation Coefficient	-0.44	0.17	-0.46	-0.39
	p value	0.03	0.63	0.02	0.06
PH	Correlation Coefficient	-0.41	0.52	-0.62	-0.20
	p value	0.04	0.12	0.001	0.35
PL	Correlation Coefficient	-0.15	-0.42	-0.19	-0.24
	p value	0.45	0.23	0.35	0.27
MTR25%	Correlation Coefficient	-0.53	0.50	-0.59	-0.41
	p value	0.005	0.14	0.002	0.04
MTR50%	Correlation Coefficient	-0.38	0.06	-0.49	-0.31
	p value	0.06	0.87	0.02	0.14
MTR75%	Correlation Coefficient	-0.13	-0.42	-0.21	-0.28
	p value	0.52	0.23	0.31	0.18
b) White matter histogram analysis					
AVMTR	Correlation Coefficient	-0.39	0.35	-0.39	-0.31
	p value	0.05	0.32	0.05	0.14
PH	Correlation Coefficient	-0.28	0.12	-0.38	-0.29
	p value	0.17	0.75	0.06	0.17
PL	Correlation Coefficient	-0.38	0.18	-0.38	-0.32
	p value	0.06	0.62	0.06	0.12
MTR25%	Correlation Coefficient	-0.39	0.12	-0.41	-0.38
	p value	0.05	0.75	0.04	0.07
MTR50%	Correlation	-0.42	0.30	-0.41	-0.30

	Coefficient				
	p value	0.03	0.40	0.04	0.15
MTR75%	Correlation Coefficient	-0.24	-0.18	-0.23	-0.27
	p value	0.23	0.62	0.27	0.20
c) Grey matter histogram analysis					
AVMTR	Correlation Coefficient	-0.32	0.000	-0.44	-0.44
	p value	0.11	0.87	0.03	0.03
PH	Correlation Coefficient	-0.55	0.23	-0.55	-0.50
	p value	0.003	0.52	0.004	0.02
PL	Correlation Coefficient	-0.22	-0.12	-0.38	-0.41
	p value	0.28	0.74	0.05	0.05
MTR25%	Correlation Coefficient	-0.39	-0.12	-0.49	-0.47
	p value	0.05	0.75	0.02	0.02
MTR50%	Correlation Coefficient	-0.28	-0.12	-0.40	-0.40
	p value	0.16	0.74	0.04	0.05
MTR75%	Correlation Coefficient	-0.18	-0.24	-0.28	-0.30
	p value	0.38	0.51	-0.16	0.16
d) ROI analysis					
Right caudate	Correlation Coefficient	-0.34	-0.29	-0.27	-0.48
	p value	0.09	0.42	0.19	0.02
Left caudate	Correlation Coefficient	-0.27	-0.17	-0.20	-0.29
	p value	0.19	0.63	0.34	0.16
Right putamen	Correlation Coefficient	-0.31	-0.29	-0.27	-0.13
	p value	0.13	0.42	-0.19	0.54
Left putamen	Correlation Coefficient	-0.21	-0.52	-0.24	-0.50
	p value	0.31	0.12	0.24	0.02
Right pulvinar	Correlation Coefficient	-0.22	-0.53	-0.22	-0.27
	p value	0.28	0.12	0.28	0.21
Left pulvinar	Correlation Coefficient	-0.24	-0.52	-0.34	-0.38
	p value	0.24	0.12	0.09	0.06

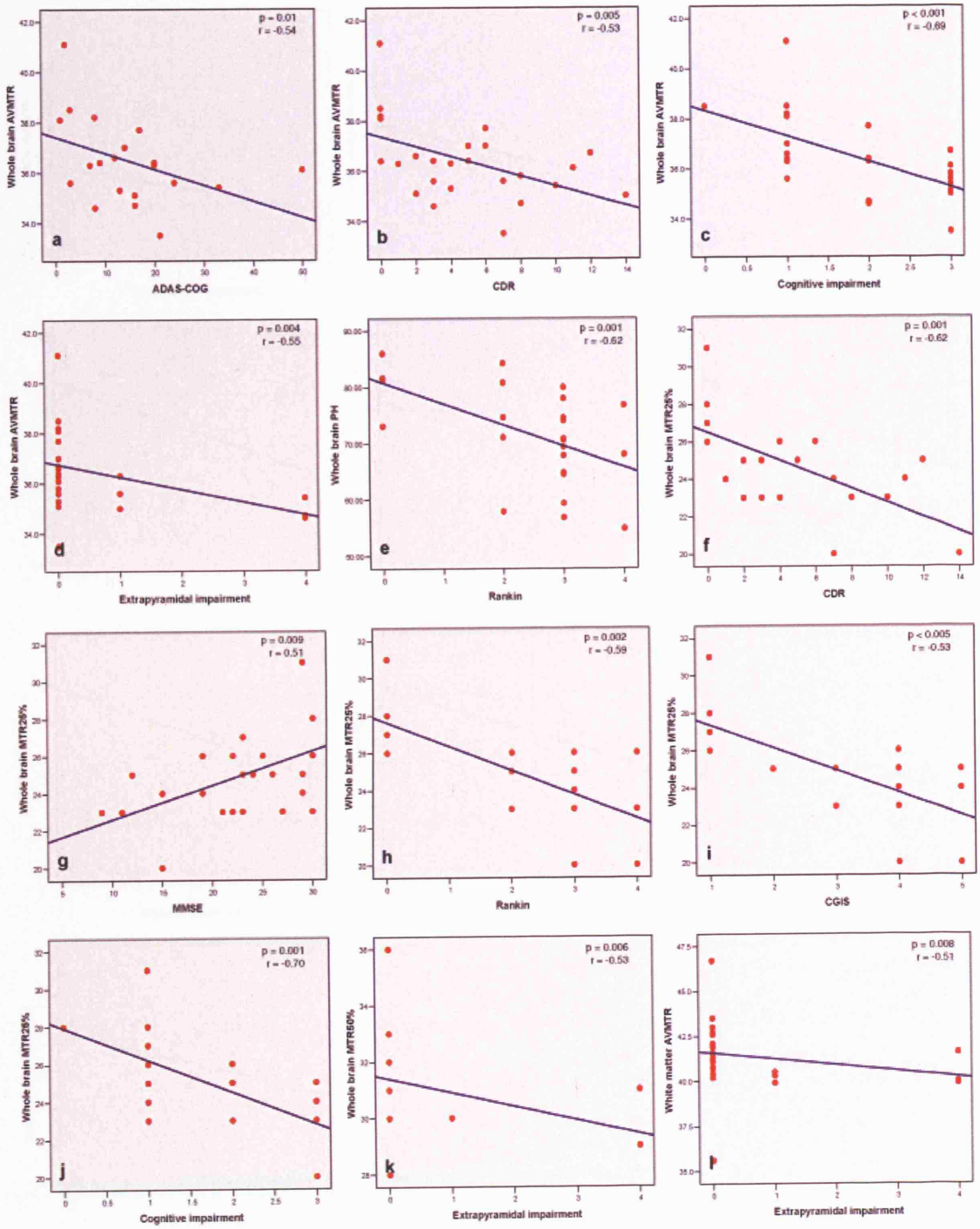
Table 10: Correlations between MT measures and videoed cognitive and motor scores at baseline in all patients

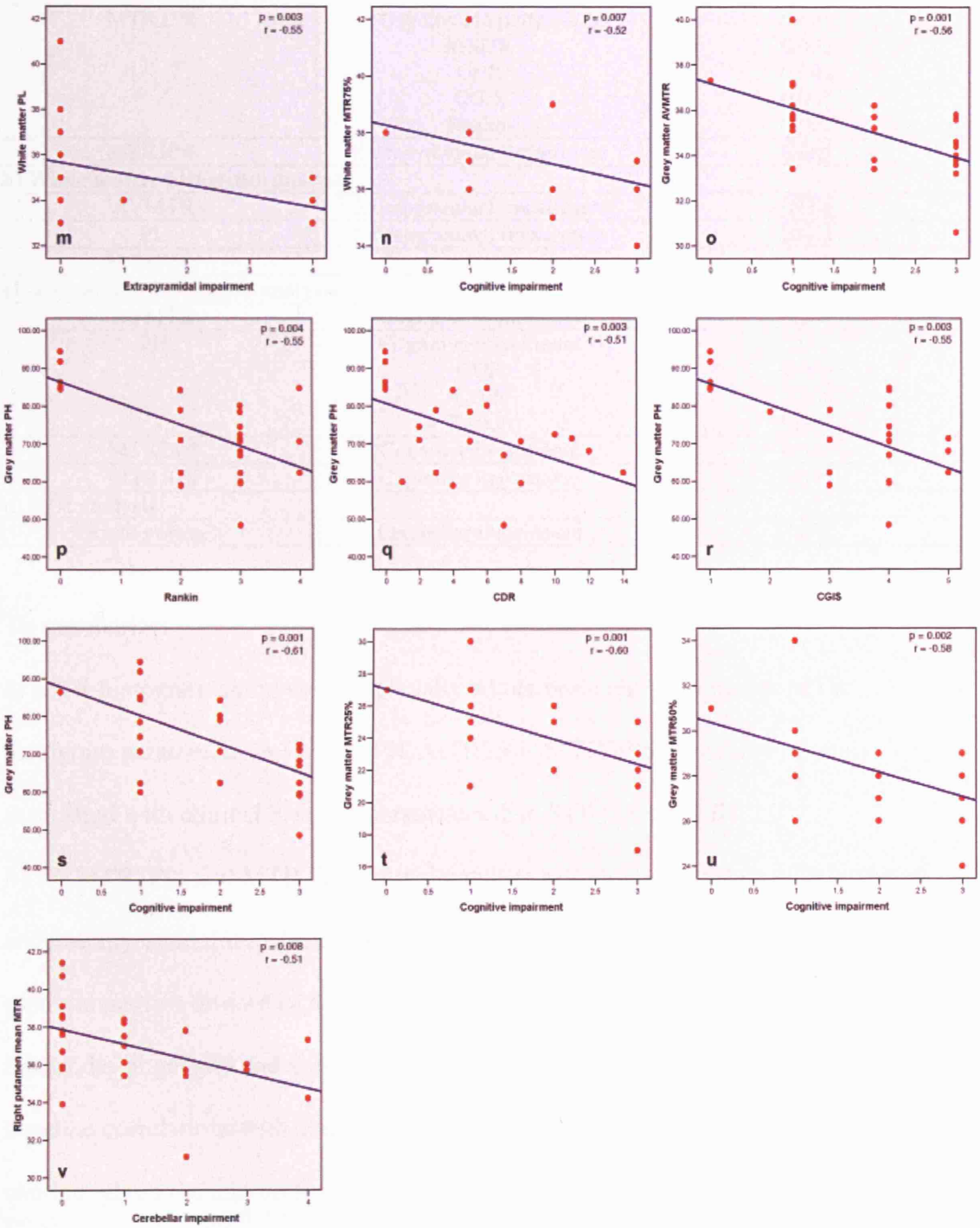
MT measures		Cognitive impairment	Extrapyramidal impairment	Pyramidal impairment	Cerebellar impairment
a) Whole brain histogram analysis					
AVMTR	Correlation Coefficient	-0.69	-0.55	-0.32	-0.009
	p value	<0.001	0.004	0.11	0.96
PH	Correlation Coefficient	-0.28	-0.41	-0.1	-0.21
	p value	0.16	0.04	0.64	0.30
PL	Correlation	-0.11	-0.34	-0.31	-0.14

	Coefficient				
	p value	0.58	0.09	0.12	0.48
MTR25%	Correlation Coefficient	-0.70	-0.49	-0.33	-0.09
	p value	<0.001	0.01	0.10	0.68
MTR50%	Correlation Coefficient	-0.46	-0.53	-0.32	-0.18
	p value	0.01	0.006	0.10	0.38
MTR75%	Correlation Coefficient	-0.31	-0.30	-0.29	0.02
	p value	0.12	0.14	0.16	0.92
b) White matter histogram analysis					
AVMTR	Correlation Coefficient	-0.44	-0.51	-0.350	-0.30
	p value	0.023	0.008	0.08	0.13
PH	Correlation Coefficient	-0.08	-0.28	-0.25	-0.40
	p value	0.70	0.17	0.21	0.04
PL	Correlation Coefficient	-0.44	-0.55	-0.44	-0.27
	p value	0.02	0.003	0.03	0.18
MTR25%	Correlation Coefficient	-0.41	-0.48	-0.38	-0.31
	p value	0.04	0.01	0.06	0.13
MTR50%	Correlation Coefficient	-0.43	-0.48	-0.35	-0.31
	p value	0.03	0.01	0.08	0.13
MTR75%	Correlation Coefficient	-0.52	-0.31	-0.30	-0.07
	p value	0.007	0.12	0.14	0.74
c) Grey matter histogram analysis					
AVMTR	Correlation Coefficient	-0.60	-0.35	-0.16	0.002
	p value	0.001	0.08	0.44	0.99
PH	Correlation Coefficient	-0.61	-0.37	-0.30	-0.12
	p value	0.001	0.06	0.14	0.55
PL	Correlation Coefficient	-0.44	-0.21	-0.27	-0.06
	p value	0.03	0.30	0.19	0.77
MTR25%	Correlation Coefficient	-0.60	-0.45	-0.27	-0.04
	p value	0.001	0.02	0.18	0.84
MTR50%	Correlation Coefficient	-0.58	-0.34	-0.13	0.04
	p value	0.002	0.09	0.52	0.86
MTR75%	Correlation Coefficient	-0.48	-0.29	-0.20	0.03
	p value	0.01	0.16	0.33	0.90
d) ROI analysis					
Right caudate	Correlation Coefficient	-0.38	-0.16	-0.44	-0.33

	p value	0.06	0.43	0.02	0.10
Left caudate	Correlation Coefficient	-0.35	-0.07	-0.01	-0.10
	p value	0.08	0.75	0.96	0.64
Right putamen	Correlation Coefficient	-0.10	-0.21	-0.31	-0.51
	p value	0.63	0.32	0.12	0.008
Left putamen	Correlation Coefficient	-0.28	-0.06	0.03	-0.10
	p value	0.17	0.77	0.87	0.63
Right pulvinar	Correlation Coefficient	-0.08	-0.31	-0.29	-0.22
	p value	0.68	0.12	0.15	0.28
Left pulvinar	Correlation Coefficient	-0.04	-0.24	-0.07	-0.12
	p value	0.85	0.24	0.72	0.56

Figure 58: Significant ($p<0.01$) correlations between MT measures and clinical scores, a-k: correlations for whole brain histogram parameters, l-n: correlations for white matter histogram parameters, o-u: correlations for grey matter histogram parameters, v: correlation for right putaminal mean MTR. All associations are low MT measures associated with poorer function (see p values for specific details of scales)


p = 0.01
r = -0.54
Whole brain AVMTR
ADAS-COG
a
p = 0.005
r = -0.53
Whole brain AVMTR
CDR
b
p < 0.001
r = -0.69
Whole brain AVMTR
Cognitive impairment
c
p = 0.004
r = -0.55
Whole brain AVMTR
Extrapyramidal impairment
d
p = 0.001
r = -0.62
Whole brain PH
Rankin
e
p = 0.001
r = -0.62
Whole brain MTR25%
CDR
f
p = 0.009
r = 0.51
Whole brain MTR25%
MMSE
g
p = 0.002
r = -0.59
Whole brain MTR25%
Rankin
h
p < 0.005
r = -0.53
Whole brain MTR25%
CGIS
i
p = 0.001
r = -0.70
Whole brain MTR25%
Cognitive impairment
j
p = 0.006
r = -0.53
Whole brain MTR50%
Extrapyramidal impairment
k
p = 0.008
r = -0.51
White matter AVMTR
Extrapyramidal impairment
l



The above associations are summarised in Table 11.

Table 11: Summary of significant associations between MT measures and clinical scores (note: grey shaded p values indicate associations which should be interpreted with caution, given the scatterplots)

MTR values	Clinical scores	p value
a) Whole brain histogram analysis		
AVMTR	Cognitive impairment	<0.001
	Extrapyramidal impairment	*0.004*
	ADAS-COG	0.01
	CDR	0.005
PH	Rankin	0.001

MTR25%	Cognitive impairment MMSE CDR CGIS Rankin	<0.001 0.009 0.001 0.005 0.002
MTR50%	Extrapyramidal impairment	***0.006***
b) White matter histogram analysis		
AVMTR	Extrapyramidal impairment	***0.008***
PL	Extrapyramidal impairment	***0.003***
MTR75%	Cognitive impairment	0.007
c) Grey matter histogram analysis		
AVMTR	Cognitive impairment	0.001
PH	Cognitive impairment CDR CGIS Rankin	0.001 0.003 0.003 0.004
MTR25%	Cognitive impairment	0.001
MTR50%	Cognitive impairment	0.002
d) ROI analysis		
Right putamen	Cerebellar impairment	0.008

To summarise:

a) MTR histogram parameters, especially whole brain and grey matter MTR histogram parameters (AVMTR, PH, MTR25%, MTR50%), are more strongly associated with clinical baseline parameters than ROI mean MTRs.

b) AVMTR, PL, and MTR 75% of white matter histograms correlate with cognitive and extrapyramidal impairment alone. This may be because prion disease is predominantly a disease of the grey matter, involving the cerebellar and cortical grey matter, basal ganglia and thalami. This is reflected in the above results. However, baseline correlations with extrapyramidal impairment need to be interpreted with caution, given the non-uniform spread of observations in scatterplots d (correlation of whole brain AVMTR with extrapyramidal impairment), k (correlation of whole brain MTR50% with extrapyramidal impairment), l (correlation of white matter AVMTR with extrapyramidal impairment), and m (correlation of white matter PL with extrapyramidal impairment). These associations have been highlighted in grey in summary Table 11 above.

c) MTR histogram parameters may indicate global cerebral changes when conventional DWI/FLAIR sequences are normal, which was the case in all but four patients.

d) MTR histogram parameters may provide valuable indices of disease severity in future therapeutic trials, as they are lower in patients with lower MMSE, CDR, ADAS-COG, CGIS, Rankin, cognitive, extrapyramidal and cerebellar impairment scores. No correlations were found between MT measures and pyramidal impairment, ADL, GCS and BPRS at baseline.

e) Rankin and CDR, being global measures of cognition, appeared to be most strongly associated with baseline MT measures.

When baseline Spearman rank correlation analysis was performed between MTRs derived from ROIs and histogram measures and clinical scores in patients with normal conventional imaging (n=22), a larger number of significant ($p<0.01$) correlations were established compared to analysis with the complete cohort of 26 patients:

Table 12: Summary of significant associations between MT measures and clinical scores in patients with normal conventional imaging (n=22)

MTR values	Clinical scores	p value
a) Whole brain histogram analysis		
AVMTR	Cognitive impairment	<0.001
	Extrapyramidal impairment	0.01
	ADAS-COG	0.004
	CDR	<0.001
	CGIS	0.008
	Rankin	0.005
	MMSE	0.006
PH	Rankin	0.001
MTR25%	Cognitive impairment	<0.001
	MMSE	0.005
	CDR	0.001
	CGIS	0.002
	Rankin	0.001
	MMSE	0.005
MTR50%	Cognitive impairment	0.008
	ADAS-COG	0.008
	CDR	0.008
	Rankin	0.002
b) White matter histogram analysis		
PL	Extrapyramidal impairment	0.01
MTR25%	Rankin	0.009

MTR50%	Rankin	0.007
MTR75%	Cognitive impairment ADAS-COG	0.006 0.004
c) Grey matter histogram analysis		
AVMTR	Cognitive impairment ADAS-COG CDR Rankin	<0.001 0.01 0.002 0.01
PH	Cognitive impairment CDR CGIS Rankin	0.001 0.003 0.006 0.003
MTR25%	Cognitive impairment CDR Rankin	0.001 0.004 0.005
MTR50%	Cognitive impairment CDR	0.001 0.008
MTR75%	ADAS-COG	0.008
d) ROI analysis		
Right pulvinar	GCS	0.007

3.5.2 Relationship between baseline MT measures and other baseline factors

The best independent, baseline clinical scores predicting baseline disease progression as measured by global and regional cerebral MR magnetisation transfer ratios (MTRs) were determined from videoed and non-videoed neurological tests at baseline. This analysis was carried out in two steps:

a) Univariate linear regession, with the baseline MT measure as the dependent variable, correlated with each of the clinical scores one at a time.

b) Baseline clinical scores which reached a significance threshold of $p<0.10$ in the univariate analysis (a) were jointly considered in a multivariate model with outcome measure baseline MT measure. A backward selection approach was adopted, in which model fitting was started with all clinical variables of interest reaching $p<0.10$. Then the least significant variable was dropped (if $p>0.05$) and analysis was continued by successively re-fitting reduced models and applying the same rule until all remaining variables (if any) had a p value of <0.05. These were considered independent predictors of baseline disease state according to MT measures. If no variable reached

a p value of<0.05 in the adjusted model fitting procedure, none of the variables were considered independent predictors of disease progression.

These steps are described for different MT measures in Tables 13, 14, 15 and 16 below (note: if worsening clinical condition means the clinical score is increasing, then expected direction of association is a negative B value. Measures where decrease in the score means worsening, the expected direction of association is a positive B value. B values represent the difference in baseline MT measures associated with one unit higher baseline clinical score. All associations were observed to be in the expected direction, except associations between GCS and right and left pulvinar (Table 16), which have been marked with an asterisk (*)).

Table 13: Whole brain histogram measures as dependent variables. Significant correlations (p<0.05) on multivariate regression have been highlighted in black. In this case, Rankin (AVMTR, p=<0.001, MTR50%, p=0.001, MTR75%, p=0.05), GCS (PH, p=0.04) and MMSE (MTR25%, p=0.008) were considered independent predictors of poorer MTR at baseline status

Dependent variable	**Independent variable**	**p value**	**B value**	**95% confidence interval for B**	
				Lower bound	Upper bound
AVMTR					
a) Univariate regression	MMSE	0.03	0.10	0.13	0.20
	ADAS-COG	0.04	-0.06	-0.13	-0.003
	CDR	0.008	-0.20	-0.34	-0.06
	CGIS	0.001	-0.68	-1.07	-0.36
	Rankin	<0.001	-0.73	-1.10	-0.36
	Cognitive impairment	0.001	-1.02	-1.55	-0.49
	Extrapyramidal impairment	0.04	-0.50	-0.96	-0.04
b) Multivariate regression	Rankin	<-0.001	-0.73	-1.10	-0.36
PH					
a) Univariate regression	CGIS	0.03	-2.80	-5.20	-0.38
	GCS	0.04	14.29	1.04	27.53
	Rankin	0.002	-3.76	-5.98	-1.53
b) Multivariate regression	GCS	0.04	14.29	1.04	27.53
PL					
a) Univariate regression	Rankin	0.09	-0.44	-0.95	0.07
	Extrapyramidal impairment	0.09	-0.46	-0.99	0.08

b) Multivariate regression	No factors significant at p<0.05				
MTR25%					
a) Univariate regression	MMSE	0.008	0.18	0.05	0.31
	ADL	0.03	0.28	0.031	0.53
	ADAS-COG	0.03	-0.10	-0.18	-0.01
	CDR	0.001	-0.38	-0.58	-0.18
	CGIS	<0.001	-1.20	-1.80	-0.65
	GCS	0.1	3.40	-0.79	7.70
	Rankin	<0.001	-1.28	-1.81	-0.75
	Cognitive impairment	<0.001	-1.70	-2.50	-0.91
	Extrapyramidal impairment	0.08	-0.65	-1.40	0.10
	Cerebellar impairment	0.08	-0.66	-1.40	0.09
b) Multivariate regression	MMSE	0.008	0.18	0.05	0.31
MTR50%					
a) Univariate regression	CDR	0.06	-0.15	-0.31	0.004
	CGIS	0.003	-0.66	-1.09	-0.24
	Rankin	0.001	-0.77	-1.10	-0.37
	Cognitive impairment	0.02	-0.79	-1.43	-0.15
	Extrapyramidal impairment	0.05	-0.49	-0.99	0.006
b) Multivariate regression	Rankin	0.001	-0.77	-1.10	-0.37
MTR75%					
a) Univariate regression	Rankin	0.05	-0.41	-0.83	0.002
	Extrapyramidal impairment	0.08	-0.39	-0.83	0.05
b) Multivariate regression	Rankin	0.05	-0.41	-0.83	0.002

Table 14: White matter histogram measures as dependent variables. Significant correlations (p<0.05) on multivariate regression have been highlighted in black. In this case, cerebellar impairment (AVMTR, p=0.01, MTR25%, p=0.02), Rankin (PH, p=0.03) and cognitive impairment (MTR50%, p=0.03, MTR75%, p=0.02) were considered independent predictors of poorer MTR status at baseline

Dependent variable	**Independent variable**	**p value**	**B value**	**95% confidence interval for B**	
				Lower bound	Upper bound
AVMTR					
a) Univariate regression	CGIS	0.02	-0.64	-1.14	-0.13
	Rankin	0.01	-0.66	-1.17	-0.15
	Cognitive impairment	0.02	-0.85	-1.59	-0.12
	Cerebellar	0.01	-0.69	-1.23	-0.16

	impairment				
b) Multivariate regression	Cerebellar impairment	0.01	-0.69	-1.23	-0.16
PH					
a) Univariate regression	CGIS	0.08	-6.59	-13.94	0.76
	Rankin	0.03	-7.87	-15.00	-0.67
	Cerebellar impairment	0.05	-7.86	-15.50	-0.18
b) Multivariate regression	Rankin	0.03	-7.87	-15.00	-0.67
PL					
a) Univariate regression	CGIS	0.01	-0.60	-1.06	-0.15
	Rankin	0.008	-0.63	-1.08	-0.18
	Cognitive impairment	0.04	-0.70	-1.38	-0.03
	Extrapyramidal impairment	0.06	-0.49	-1.00	0.02
	Pyramidal impairment	0.07	-0.55	-1.16	0.05
	Cerebellar impairment	0.09	-0.45	-0.97	0.07
b) Multivariate regression	No factors independently significant at $p<0.05$				
MTR25%					
a) Univariate regression	CGIS	0.02	-0.85	-1.53	-0.17
	Rankin	0.01	-0.85	-1.56	-0.21
	Cognitive impairment	0.04	-1.06	-2.06	-0.07
	Cerebellar impairment	0.02	-0.89	-1.6	-0.16
b) Multivariate regression	Cerebellar impairment	0.02	-0.89	-1.6	-0.16
MTR50%					
a) Univariate regression	CDR	0.06	-0.17	-0.35	0.01
	CGIS	0.004	-0.73	-1.20	-0.25
	Rankin	0.004	-0.74	-1.22	-0.25
	Cognitive impairment	0.03	-0.83	-1.56	-0.10
	Cerebellar impairment	0.03	-0.62	-1.16	-0.07
b) Multivariate regression	Cognitive impairment	0.03	-0.83	-1.56	-0.10
MTR75%					
a) Univariate regression	CGIS	0.05	-0.42	-0.84	-0.009
	Rankin	0.03	-0.45	-0.86	-0.04
	Cognitive impairment	0.02	-0.68	-1.26	-0.11
b) Multivariate regression	Cognitive impairment	0.02	-0.68	-1.26	-0.11

Table 15: Grey matter histogram measures as dependent variables. Significant correlations ($p<0.05$) on multivariate regression have been highlighted in black. In this case, cognitive impairment (AVMTR,

p=0.001, MTR25%, p=0.002, MTR50%, p=0.004, MTR 75%, p=0.03), and Rankin (PH, p=<0.001, PL, p=0.004) were considered independent predictors of poorer MTR status at baseline

Dependent variable	Independent variable	p value	B value	95% confidence interval for B	
				Lower bound	Upper bound
AVMTR					
a) Univariate regression	MMSE	0.05	0.11	-0.002	0.22
	CDR	0.03	-0.19	-0.35	-0.02
	CGIS	0.01	-0.64	-1.11	-0.16
	Rankin	0.003	-0.73	-1.19	-0.27
	Cognitive impairment	0.001	-1.09	-1.72	-0.47
b) Multivariate regression	Cognitive impairment	0.001	-1.09	-1.72	-0.47
PH					
a) Univariate regression	MMSE	0.02	0.86	0.16	1.57
	CDR	0.008	-1.49	-2.56	-0.43
	CGIS	<0.001	-5.62	-8.40	-2.83
	Rankin	<0.001	-5.64	-8.46	-2.82
	BPRS	0.06	-0.72	-1.48	0.04
	Cognitive impairment	0.001	-7.56	-11.68	-3.45
b) Multivariate regression	Rankin	<0.001	-5.64	-8.46	-2.82
PL					
a) Univariate regression	CGIS	0.03	-0.55	-1.04	-0.06
	Rankin	0.004	-0.70	-1.17	-0.25
	Cognitive impairment	0.03	-0.77	-1.47	-0.07
b) Multivariate regression	Rankin	0.004	-0.70	-1.17	-0.25
MTR25%					
a) Univariate regression	MMSE	0.06	0.15	-0.009	0.31
	CDR	0.05	-0.24	-0.49	0.003
	CGIS	0.01	-0.90	-1.59	-0.21
	Rankin	0.004	-1.03	-1.69	-0.36
	Cognitive impairment	0.002	-1.56	-2.47	-0.65
b) Multivariate regression	Cognitive impairment	0.002	-1.56	-2.47	-0.65
MTR50%					
a) Univariate regression	MMSE	0.09	0.11	-0.02	0.23
	CDR	0.09	-0.16	-0.36	0.03
	CGIS	0.03	-0.62	-1.16	-0.08
	Rankin	0.006	-0.75	-1.27	-0.24
	Cognitive impairment	0.004	-1.10	-1.81	-0.38
b) Multivariate regression	Cognitive impairment	0.004	-1.10	-1.81	-0.38
MTR75%					
a) Univariate	CGIS	0.08	-0.39	-0.83	0.05

regression					
	Rankin	0.03	-0.48	-0.92	-0.05
	Cognitive impairment	0.03	-0.70	-1.30	-0.09
b) Multivariate regression	Cognitive impairment	0.03	-0.70	-1.30	-0.09

Table 16: ROI MTRs as dependent variables. Significant correlations ($p<0.05$) on multivariate regression have been highlighted in black. In this case, pyramidal impairment (right caudate, p=0.03), MMSE (left caudate, p=0.05), CGIS (right putamen, p=0.03), and GCS (right pulvinar, p=0.03) were considered independent predictors of poorer MTR status at baseline. Of note, it was found that patients with better (higher) GCS had statistically significantly (p=0.03) poorer MTR status at baseline, which though unexpected, may be a false positive due to threshold effect (see Figure 71) or influence of a single outlier, as suggested by the scatterplot.

Dependent variable	**Independent variable**	**p value**	**B value**	**95% confidence interval for B**	
				Lower bound	Upper bound
Right caudate					
a) Univariate regression	CGIS	0.08	-0.61	-1.31	0.09
	BPRS	0.08	-0.14	-0.30	0.02
	Cognitive impairment	0.06	-0.95	-1.94	0.03
	Pyramidal impairment	0.03	-0.96	-1.80	-0.12
b) Multivariate regression	Pyramidal impairment	0.03	-0.96	-1.80	-0.12
Left caudate					
a) Univariate regression	MMSE	0.05	0.16	-0.003	0.32
	ADAS-COG	0.08	-0.09	-0.19	0.01
	Cognitive impairment	0.05	-1.01	-2.04	0.01
b) Multivariate regression	MMSE	0.05	0.16	-0.003	0.32
Right putamen					
a) Univariate regression	CGIS	0.03	-0.72	-1.33	-0.10
	Rankin	0.03	-0.72	-1.34	-0.09
	Cerebellar impairment	0.02	-0.79	-1.44	-0.13
b) Multivariate regression	CGIS	0.03	-0.72	-1.33	-0.10
Left putamen					
a) Univariate regression	CGIS	0.10	-0.51	-1.11	0.10
	Rankin	0.06	-0.58	-1.18	0.02
	BPRS	0.08	-0.12	-0.26	0.01
b) Multivariate regression	No factors significant at $p<0.05$				
Right pulvinar					
a) Univariate regression	CGIS	0.05	-0.58	-1.14	-0.01

	GCS	0.03	-3.51*	-6.50	-0.53
	Rankin	0.05	-0.57	-1.14	-0.003
b) Multivariate regression	GCS	0.03	-3.51*	-6.50	-0.53
Left pulvinar					
a) Univariate regression	CGIS	0.04	-0.62	-1.20	-0.05
	GCS	0.09	-3.20*	-6.90	0.56
	Rankin	0.02	-0.71	-1.27	-0.15
b) Multivariate regression	No factors independently significant at p<0.05				

To summarise:

a) There were no independent clinical predictors of baseline whole brain PL, white matter PL, left putamen and left pulvinar ROI MTRs.

b) The independent clinical predictors of the remaining baseline MT measures are described in Table 17.

Table 17: Summary table of independent clinical predictors of baseline MT measures

Baseline MT measures	Independent clinical predictors of baseline MT measures	p value on multivariate regression
a) Whole brain histogram parameters		
AVMTR	Rankin	<0.001
PH	GCS	0.04
MTR25%	MMSE	0.008
MTR50%	Rankin	0.001
MTR75%	Rankin	0.05
Rankin, GCS and MMSE were independent clinical predictors of baseline whole brain histogram MTR parameters (visual inspection of scatterplots does not suggest these results are driven by outliers, in case of MMSE and Rankin, though GCS may be influenced by a single outlier)		
b) White matter histogram parameters		
AVMTR	Cerebellar impairment	0.01
PH	Rankin	0.03
MTR25%	Cerebellar impairment	0.02
MTR50%	Cognitive impairment	0.03
MTR75%	Cognitive impairment	0.02
Rankin, cerebellar and cognitive impairment were independent clinical predictors of baseline white matter histogram MTR parameters (visual inspection of scatterplots does not suggest these results are driven by outliers)		
c) Grey matter histogram parameters		
AVMTR	Cognitive impairment	0.001
PH	Rankin	<0.001
PL	Rankin	0.004
MTR25%	Cognitive impairment	0.002
MTR50%	Cognitive impairment	0.004
MTR75%	Cognitive impairment	0.03
Rankin and cognitive impairment were independent clinical predictors of baseline grey matter histogram MTR parameters (visual inspection of scatterplots does not suggest these results are driven by outliers)		
d) ROI MTRs		

Right caudate	Pyramidal impairment	0.03
Left caudate	MMSE	0.05
Right putamen	CGIS	0.03
Right pulvinar	GCS*	0.03
Pyramidal impairment, MMSE, CGIS, and GCS were independent clinical predictors of baseline ROI MTRs (visual inspection of scatterplots does not suggest these results are driven by outliers), though GCS was a false positive, influenced either by threshold effect or a single outlier		

In a second stage of modelling, age, sex, disease type (inherited, sporadic, variant) and duration of symptoms at baseline were considered in addition to baseline videoed and non-videoed clinical scores in a similar multivariable model (Table 18). Duration of symptoms and sex became significant ($p<0.05$) independent predictors of clinical deterioration in some cases on multivariate regression. Where independent clinical predictors had been identified in the first stage these were consistently found not to add independent predictive information after adjusting for duration of symptoms and sex, as highlighted in Table 18. Duration of symptoms was consistently more predictive than cerebellar or cognitive impairment or even added information in case of whole brain and white matter PL and left putaminal mean MTR. Generally, cognitive and cerebellar impairment confounded with duration of symptoms, which is the better predictor.

Table 18: Summary table of independent clinical and non-clinical predictors of MT measures at baseline

Baseline MT measures	**Independent clinical and non-clinical predictors of baseline MT measures**	**p value on multivariate regression**
a) Whole brain histogram parameters		
AVMTR	Rankin	<0.001
PH	GCS	0.04
PL	Duration of symptoms (previously no predictors)	0.05
MTR25%	MMSE	0.008
MTR50%	Rankin	0.001
b) White matter histogram parameters		
AVMTR	Duration of symptoms (previously cerebellar impairment)	<0.001
PH	Sex (previously Rankin)	0.02
PL	Duration of symptoms (previously no predictors)	0.01

MTR25%	Duration of symptoms (previously cerebellar impairment)	<0.001
MTR50%	Cognitive impairment	0.03
MTR75%	Duration of symptoms (previously cognitive impairment)	0.007
c) Grey matter histogram parameters		
AVMTR	Duration of symptoms (previously cognitive impairment)	0.001
PH	Duration of symptoms (previously Rankin)	<0.001
PL	Rankin	0.004
MTR25%	Duration of symptoms (previously cognitive impairment)	<0.001
MTR50%	Duration of symptoms (previously cognitive impairment)	0.002
MTR75%	Cognitive impairment	0.03
d) ROI MTRs		
Right caudate	Duration of symptoms (previously pyramidal impairment)	0.002
Left caudate	MMSE	0.05
Right putamen	Duration of symptoms (previously CGIS)	0.002
Left putamen	Duration of symptoms (previously no predictors)	0.05
Right pulvinar	GCS	0.03

3.5.3. Relationship between decline in MT measures and baseline factors

Decline in MT measures over time and clinical scores measured at the baseline visit was considered to identify independent baseline clinical predictors of subsequent decline in MT measures:

a) The B values, expressing decline in MT measures over time (decline X units per month), were estimated for each patient. These are expressed for whole brain, grey matter and white matter MTR histogram parameters and ROIs in Appendix T; while their respective boxplots are shown in Figures 60-63. Some patients estimate decline, others estimate improvement.

Visual inspection of scatterplots of estimated decline (B values) in MT measures against estimated decline in each of the clinical factors over a given time period

identified a few outliers in estimates of change in MT measures, likely to be due to measurement artefacts at single observation points skewing estimates of overall decline. With small numbers of patients, including these outlying observations in linear regression estimates of slopes could have a substantial impact on the results obtained. Therefore, these outliers were truncated before all analysis and have been clearly indicated in the tables given in Appendix T. Note that the Spearman correlation is a ranking procedure, so these outliers were still the lowest values, and truncation does not affect Spearman analysis (Figure 59).

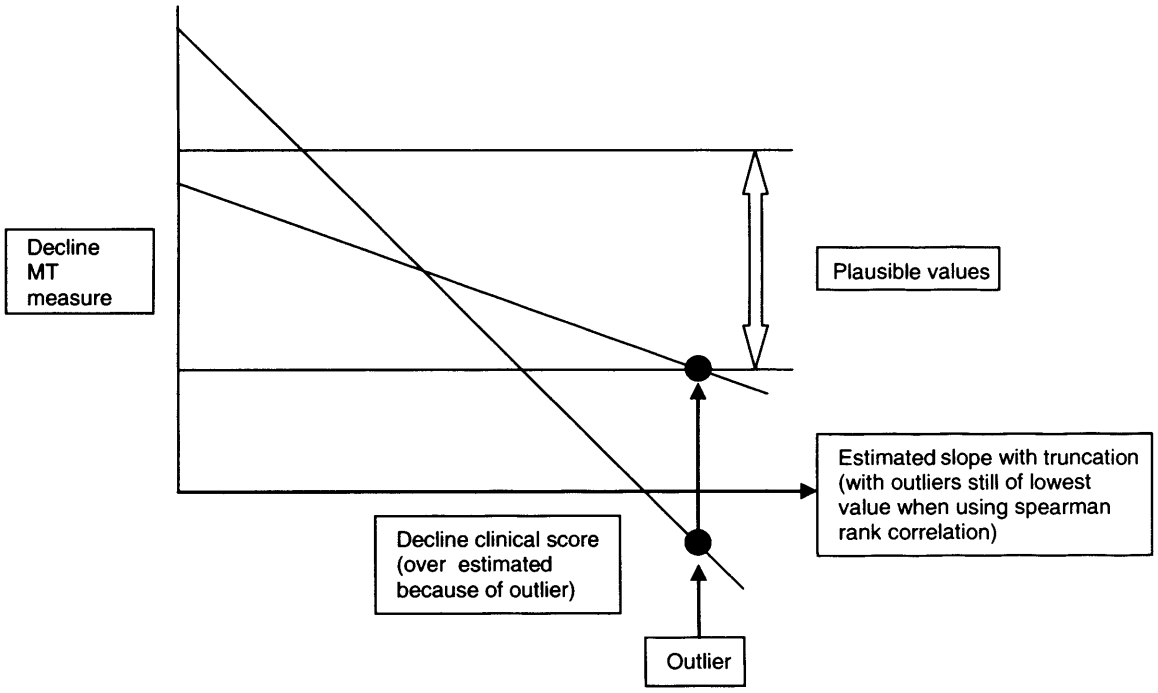


Figure 59: Effect of truncation of outliers on slopes

b) There was no evidence for a statistically significant decline over time in the group as a whole for any of the whole brain, grey matter and white matter MTR histogram parameters or mean ROI MTRs according to one-sample t-test testing slope=0. A bigger variance in whole brain, white matter and grey matter PH was observed because of variance in PH at baseline and higher PH on longitudinal analysis in

patients who underwent MRI scans under GA (GA patients also specified in tables in Appendix R).

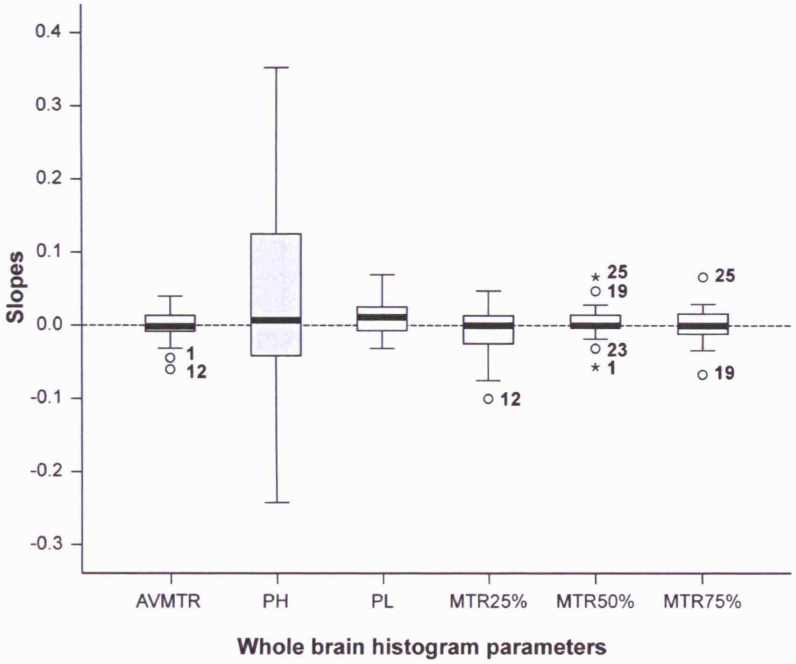


Figure 60: Boxplot showing slopes of change over time in whole brain histogram parameters

The boxplot graphs (Figures 60-66) present the data as follows:

Boxplots allow comparison of each group using a 5-number summary: the median, the 25th and 75th percentiles and the minimum and maximum observed values that are not statistically outliers. The heavy black line inside each box marks the 50th percentile, or median, of that distribution. The lower and upper hinges, or box boundaries, mark the 25th (1st quartile) and 75th percentiles (3rd quartile) of each distribution, respectively. The interquartile range (IQR) is calculated by subtracting the 1st quartile from the 3rd quartile. Whiskers appear above and below the hinges. Whiskers are vertical lines ending in horizontal lines at the largest and smallest observed values that are not statistical outliers. Any data observation which lies more than 1.5 times IQR lower than the 1st quartile or 1.5 times higher than the 3rd quartile is considered an outlier (patient number

indicated on the boxplot). Mild outliers are identified with an O, which lie between 1.5 to 3 times the IQR from the 1st and 3rd quartile and extreme values are marked with an asterisk (*), which lie more than three times the IQR from the 1st and 3rd quartiles. Patient number is mentioned next to the O or (*) in each case.

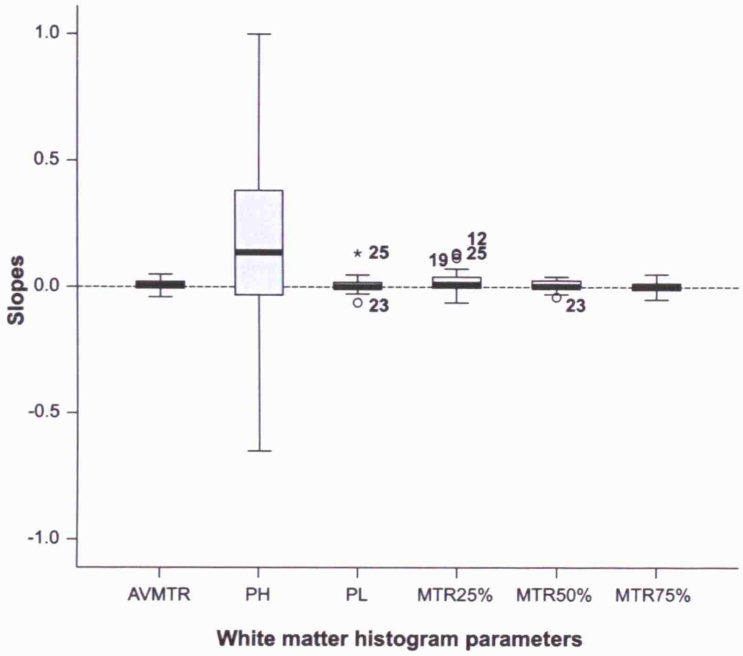


Figure 61: Boxplot showing slopes of change over time in white matter histogram parameters

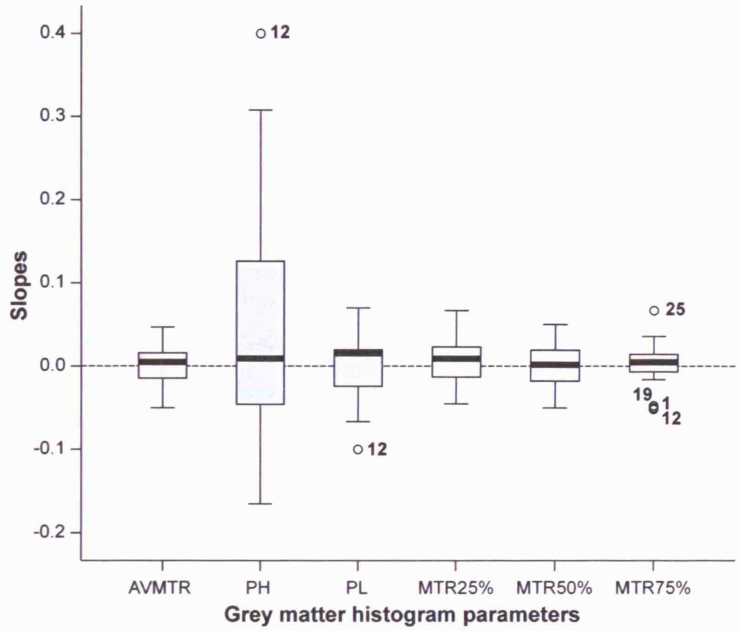


Figure 62: Boxplot showing slopes of change over time in grey matter histogram parameters

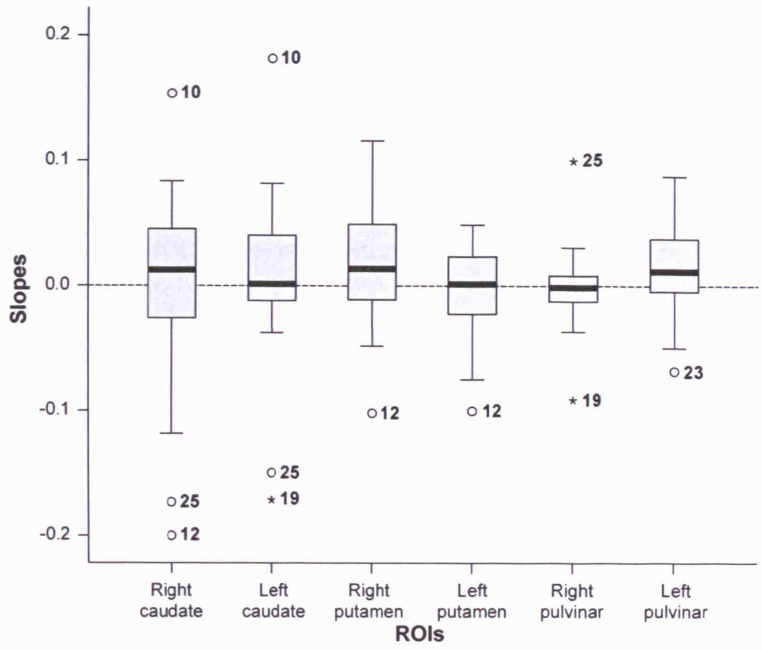


Figure 63: Box plot showing slopes of change over time in mean ROI MTRs

c) There was also no statistically significant association between decline in MTR histogram parameters or mean ROI MTRs and any baseline clinical scores. Thus none of the baseline clinical scores could be considered as independent predictors of subsequent decline in MT measures.

3.5.4 Relationship between decline in MT measures and decline in other factors

a) Linear decline in each score from neurological and psychiatric examination (BPRS, GCS, Barthel ADL, Rankin, MMSE, ADAS-COG, CDR, Rankin, CGIS and videoed cognitive and motor scores) was also estimated by estimating the B value or slope. This analysis was based on a linear regression model including all clinical measures for that patient (outcome variable), over the time period in which MT measures were measured and time (measured from baseline MT visit) as an explanatory variable. The slopes from this analysis, describing unit change per month, are described in Table 19 (slopes of change over time in ADL, CDR, CGIS and Rankin) and Appendix U (slopes of change over time in MMSE, ADAS-COG, GCS, BPRS and videoed

cognitive and motor scores). See p values for description of change in each parameter associated with worsening. Their respective boxplots are shown in Figures 64-66. Only ADL, CDR, CGIS and Rankin showed a statistically significant ($p<0.05$) decline over time in each group, for any of the above clinical scores, according to one-sample t-test testing and the null hypothesis that the slope was 0 (Table 19).

Table 19: Slopes of decline over time in ADL, CDR, CGIS and Rankin

	Barthel ADL	**CDR**	**CGIS**	**Rankin**
Pt 1	-0.02	0.05	0.03	0.000
Pt 5	0.000	0.000	0.000	0.000
Pt 6	-0.12	0.04	0.01	0.009
Pt 7	-0.03	0.04	0.01	0.000
Pt 8	-0.08	0.000	0.01	0.007
Pt 9	0.00	0.000	0.000	0.000
Pt 10	-0.07	0.07	0.000	0.02
Pt 11	-0.10	0.08	0.02	0.008
Pt 12	0.000	0.000	0.000	0.000
Pt 13	-0.02	0.03	0.008	0.008
Pt 14	-0.05	0.05	0.000	0.02
Pt 15	0.000	0.04	0.000	0.000
Pt 17	-0.21	0.05	0.003	0.000
Pt 19	-0.22	0.18	0.000	0.000
Pt 20	0.04	-0.06	-0.02	-0.01
Pt 21	-0.07	0.03	0.000	0.01
Pt 22	-0.11	0.04	0.02	0.02
Pt 23	0.000	0.06	0.000	0.000
Pt 25	-0.07	-0.07	0.000	0.000
Pt 26	0.000	0.000	0.000	0.000
Mean	-0.06	0.03	0.005	0.004
SD	0.07	0.05	0.01	0.008
Median	-0.04	0.04	0.001	0.001
25th percentile	-0.10	0.000	0.001	0.001
75th percentile	0.000	0.05	0.01	0.009
p values from one-sample t-test	0.002	0.02	0.04	0.02

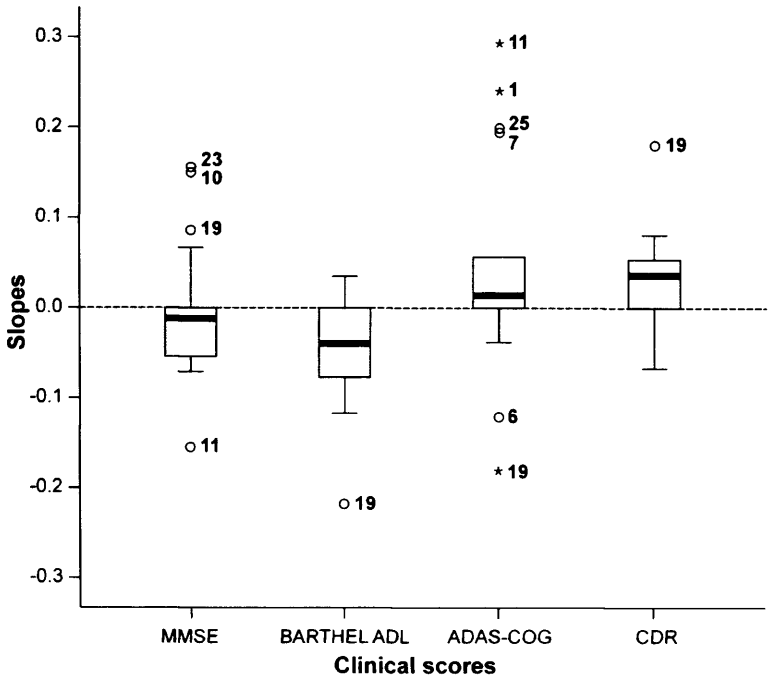


Figure 64: Boxplot showing slopes of change over time in MMSE, ADL, ADAS-COG and CDR

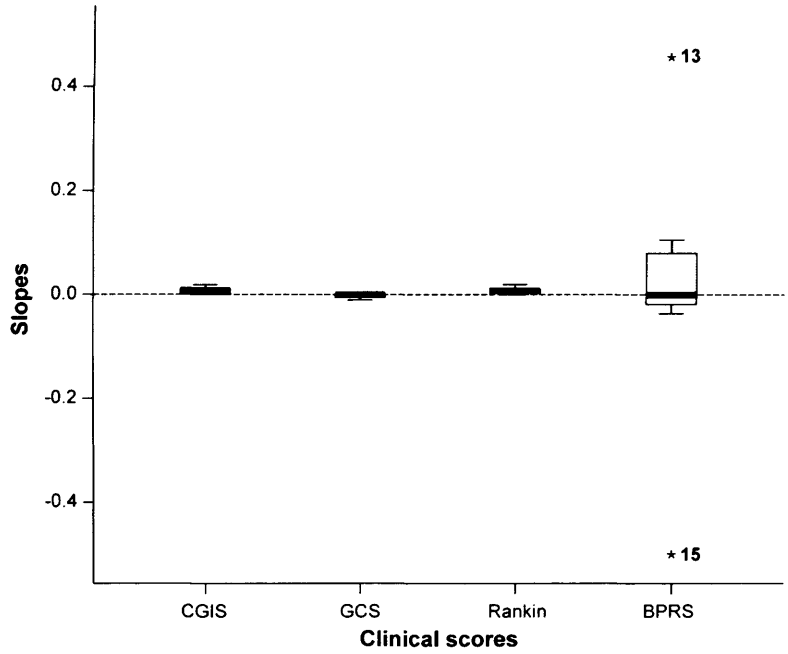


Figure 65: Boxplot showing slopes of change over time in CGIS, GCS, Rankin and BPRS

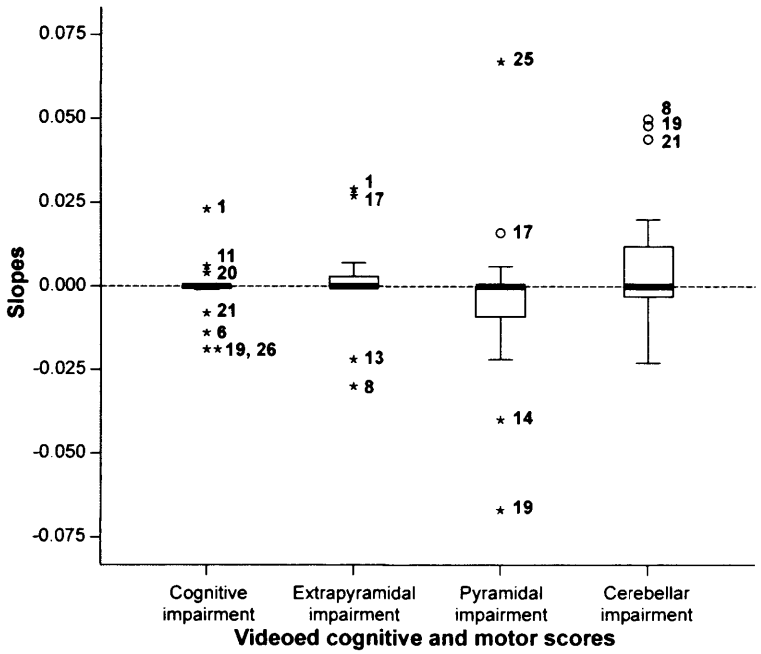


Figure 66: Boxplot showing slopes of change over time in videoed cognitive and motor scores

When the relationship between decline in clinical scores and baseline MTR measures was assessed using linear regression analysis, significant ($p<0.05$) correlations obtained were as follows:

Table 20: Significant ($p<0.05$) correlations between decline in clinical scores and baseline MTR measures

Decline clinical scores	Baseline MT measures	p value
MMSE	Grey matter PL	0.02
	R caudate MTR	0.03
ADL	Grey matter PH	0.04
	R putamen MTR	0.04
ADAS-COG	Grey matter AVMTR	0.03
	Grey matter MTR25%	0.04
	Grey matter MTR50%	0.02
	L caudate MTR	0.02
	L putamen MTR	0.02
CGIS	Grey matter PL	0.03
	Grey matter MTR50%	0.03
Extrapyramidal impairment	R pulvinar MTR	0.04
Pyramidal impairment	R putamen MTR	0.04

To summarise, grey matter MTR histogram measures (AVMTR, PH, PL, MTR25%, MTR50%) were independent predictors of subsequent decline in MMSE, ADL, ADAS-COG and CGIS, and caudate, pulvinar and putamen mean

ROI MTRs were independent predictors of subsequent decline in MMSE, ADL, ADAS-COG, extrapyramidal and pyramidal impairment.

b) Decline in MT measures versus decline in clinical scores over time

It was expected that a greater decline in MT measures would be associated with a greater decline in clinical scores. There were no significant associations between decline in MT measures and decline in MMSE, ADL, ADAS-COG, CGIS, Rankin BPRS and cognitive and cerebellar impairment. This data is described in Appendix V. Associations between decline in MT measures and decline in CDR, GCS, extrapyramidal and pyramidal impairment, including significant associations, are described in Tables 21-24. Some associations were in the unexpected direction and these have been indicated by an asterisk (*) in Appendix V and Tables 21-24.

1) Decline in MT measures versus decline in CDR (Table 21)

It was observed that the greater the decline in MT measures, the greater the decline in CDR, when decline in whole brain MTR75%, white matter MTR50% and MTR75%, grey matter MTR50% and MTR75%, and right pulvinar mean MTR was estimated against decline in CDR. These trends were statistically significant ($p<0.05$). They are highlighted in Table 21 and described with the help of graphs in Figure 67 below. As this is less likely to have occurred by chance alone, they may be more important than other MTR histogram parameters in monitoring decline. The remaining MT measures did not show a similar, statistically significant, decline with CDR.

Table 21: Associations between decline in MT measures and decline in CDR

Decline CDR	Decline MT measures	p value	Slope (B value)
	a) Whole brain histogram analysis		
	AVMTR	0.20	-0.14
	PH	0.17*	0.95

	PL	0.98*	0.003
	MTR25%	0.63*	0.08
	MTR50%	0.56	-0.07
	MTR75%	0.001	-0.41
	b) White matter histogram analysis		
	AVMTR	0.43	-0.09
	PH	0.30*	2.04
	PL	0.16	-0.24
	MTR25%	0.65	-0.11
	MTR50%	0.03	-0.20
	MTR75%	0.002	-0.31
	c) Grey matter histogram analysis		
	AVMTR	0.08	-0.18
	PH	0.11*	1.06
	PL	0.20*	0.28
	MTR25%	0.15	-0.18
	MTR50%	0.02	-0.26
	MTR75%	0.01	-0.30
	d) ROI analysis		
	Right caudate	0.66*	0.17
	Left caudate	0.62	-0.17
	Right putamen	0.99	-0.004
	Left putamen	0.56	-0.10
	Right pulvinar	<0.001	-0.50
	Left pulvinar	0.13	-0.26

*Associations in the unexpected direction

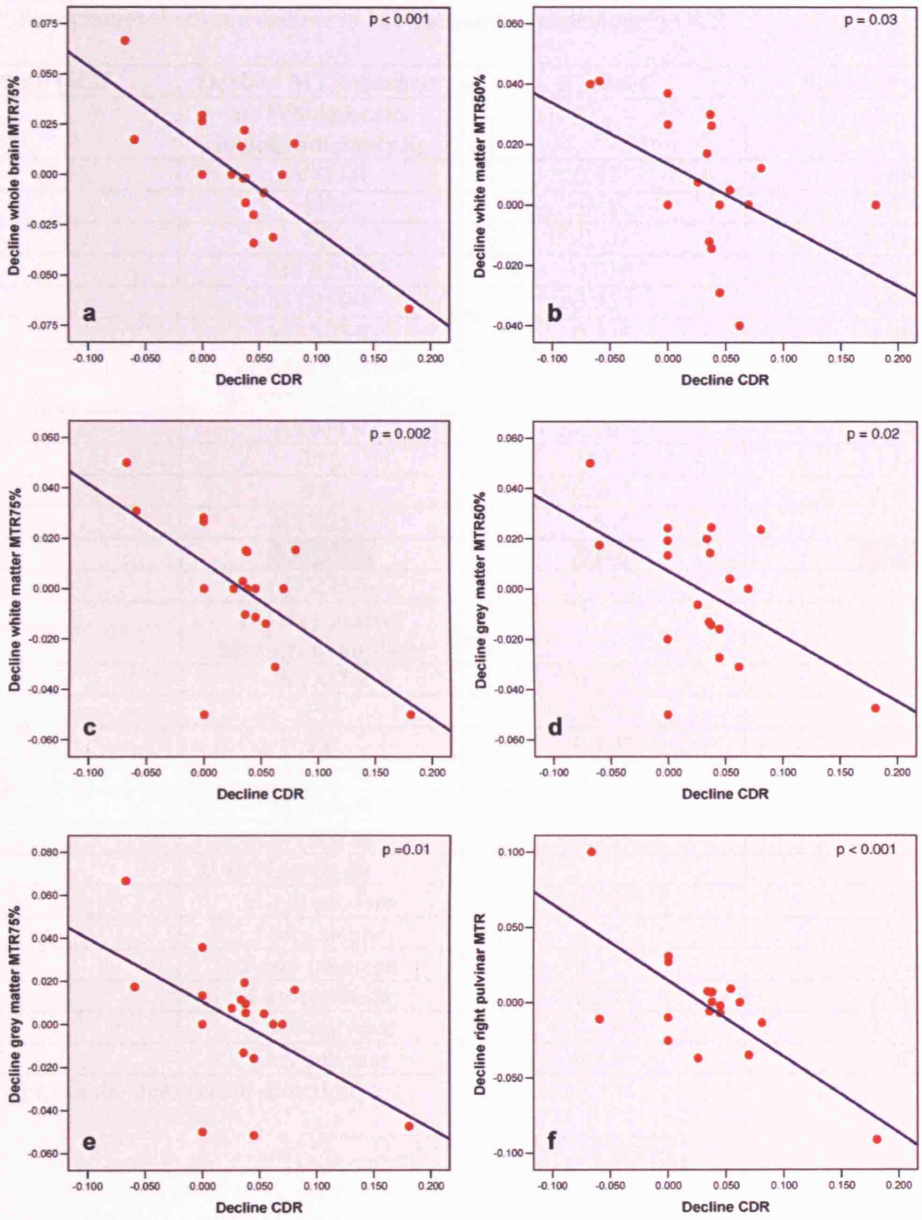


Figure 67: Graphs showing significant associations between decline in MT measures and decline in CDR

2) Decline in MT measures versus decline in GCS (Table 22)

White matter histogram MTR50% showed a statistically significant decline with improving GCS (p=0.01, Figure 68) which though unexpected may be a spurious finding because of the threshold effect (see Figure 71). There were no other significant associations.

Table 22: Associations between decline in MT measures and decline in GCS

Decline GCS	Decline MT measures	p values	Slope (B value)
	a) Whole brain histogram analysis		
	AVMTR	0.42*	-1.88
	PH	0.06	18.77
	PL	0.10	0.01
	MTR25%	0.51*	-2.34
	MTR50%	0.45*	-0.95
	MTR75%	0.34*	-1.08
	b) White matter histogram analysis		
	AVMTR	0.06*	-3.94
	PH	0.31	30.38
	PL	0.10*	-2.10
	MTR25%	0.54*	-2.46
	MTR50%	0.01*	-2.80
	MTR75%	0.12*	-3.07
	c) Grey matter histogram analysis		
	AVMTR	0.26*	-2.33
	PH	0.24	15.15
	PL	0.10*	-0.03
	MTR25%	0.06*	-2.65
	MTR50%	0.23*	-2.69
	MTR75%	0.19*	-2.79
	d) ROI analysis		
	Right caudate	0.78*	-2.52
	Left caudate	0.85	1.18
	Right putamen	0.58*	-2.92
	Left putamen	0.50*	-2.70
	Right pulvinar	0.09*	-2.93
	Left pulvinar	0.25*	-2.98

*Associations in the unexpected direction

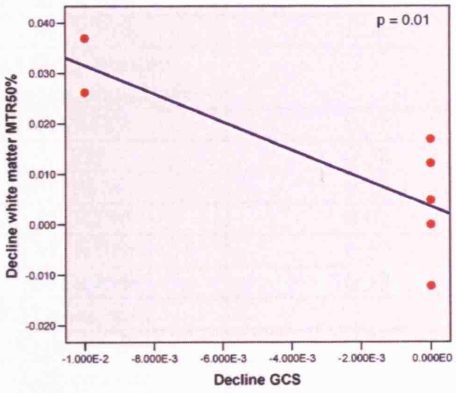


Figure 68: Graph showing significant association between decline in white matter histogram MTR50% and decline in GCS (in the unexpected direction)

3) Decline in MT measures versus decline in extrapyramidal impairment (Table 23)

It was observed that the greater the decline in extrapyramidal impairment, the greater the decline in MT measures, when decline in whole brain PH, MTR25% and MTR50% was estimated against decline in extrapyramidal impairment. These associations (which reach significance level of $p<0.05$) are highlighted in Table 23 but need to be interpreted with caution, given the scatterplots (Figure 69), as they may have been influenced by outliers. The remaining MT measures did not show a similar decline with extrapyramidal impairment.

Table 23: Associations between decline in MT measures and decline in extrapyramidal impairment

Decline extrapyramidal impairment	Decline MT measures	p value	Slope (B value)
	a) Whole brain histogram analysis		
	AVMTR	0.07	-0.69
	PH	0.03	-5.36
	PL	0.06	-0.82
	MTR25%	0.002	-1.72
	MTR50%	0.007	-1.06
	MTR75%	0.72	-0.16
	b) White matter histogram analysis		
	AVMTR	0.14	-0.58
	PH	0.15	-10.01
	PL	0.10	-0.99
	MTR25%	0.10	-1.37
	MTR50%	0.16	-0.50
	MTR75%	0.78*	0.12
	c) Grey matter histogram analysis		
	AVMTR	0.32	-0.38
	PH	0.38	-2.11
	PL	0.18	-0.88
	MTR25%	0.07	-0.80
	MTR50%	0.69	-0.17
	MTR75%	0.38	-0.40
	d) ROI analysis		
	Right caudate	0.77	-0.42
	Left caudate	0.40*	1.01
	Right putamen	0.25	-0.93
	Left putamen	0.39*	0.58
	Right pulvinar	0.63*	0.28
	Left pulvinar	0.26*	0.70

*Associations in the unexpected direction

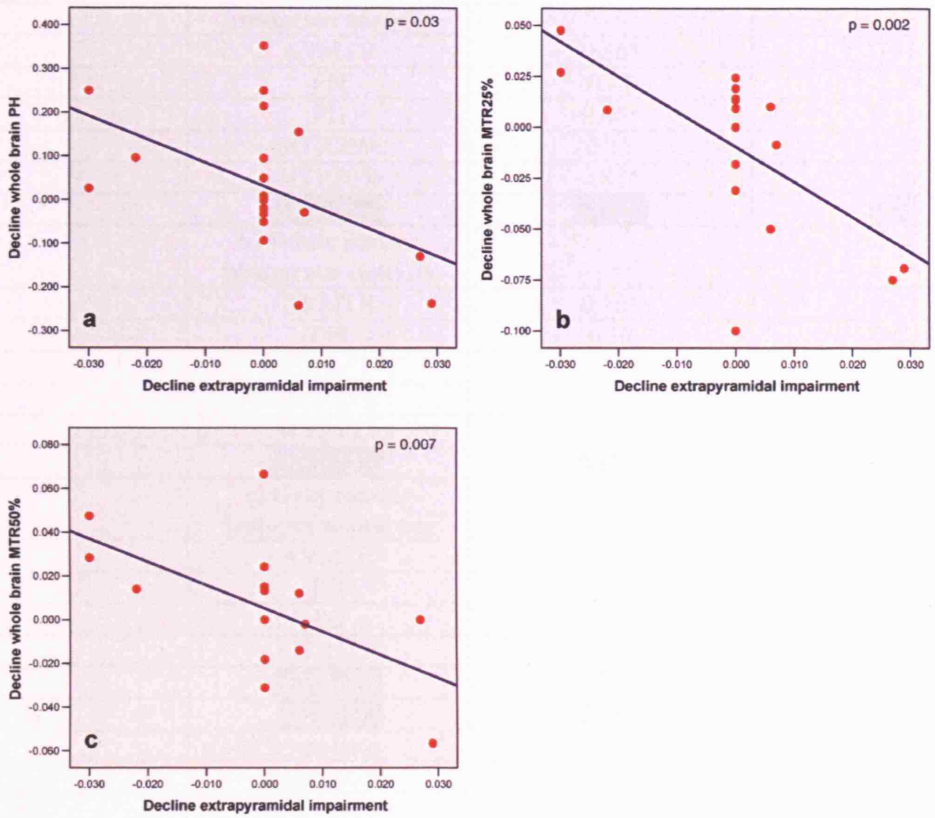


Figure 69: Graphs showing significant associations (p<0.05) between decline in MT measures and decline in extrapyramidal impairment

4) Decline in MT measures versus decline in pyramidal impairment (Table 24)

Whole brain histogram MTR75% (p=0.001), white matter histogram MTR75% (p=0.007), grey matter histogram MTR50% (p=0.05) and MTR75% (p=0.02) and right pulvinar mean MTR (p<0.001) showed a statistically significant decline with improving pyramidal impairment, which was unexpected. These trends have been highlighted in Table 24 and all associations (both significant and non-significant) in the unexpected direction have been indicated by an asterisk (*). These significant associations also need to be interpreted with caution, given the scatterplots (Figure 70), as they have been influenced by outliers.

Table 24: Associations between decline in MT measures and decline in pyramidal impairment

Decline pyramidal impairment	Decline MT measures	p value	Slope (B value)
	a) Whole brain		

	histogram analysis		
	AVMTR	0.36*	0.21
	PH	0.11	-2.32
	PL	0.66*	0.12
	MTR25%	0.15	-0.52
	MTR50%	0.81*	0.06
	MTR75%	0.001*	0.74
	b) White matter histogram analysis		
	AVMTR	0.76*	0.07
	PH	0.19	-5.35
	PL	0.19*	0.47
	MTR25%	0.79*	0.14
	MTR50%	0.38*	0.19
	MTR75%	0.007*	0.59
	c) Grey matter histogram analysis		
	AVMTR	0.13*	0.33
	PH	0.12	-2.17
	PL	0.06	-0.69
	MTR25%	0.32*	0.26
	MTR50%	0.05*	0.48
	MTR75%	0.02*	0.58
	d) ROI analysis		
	Right caudate	0.30*	-0.85
	Left caudate	0.88*	0.11
	Right putamen	0.95*	0.03
	Left putamen	0.24*	0.45
	Right pulvinar	<0.001*	1.05
	Left pulvinar	0.08*	0.61

*Associations in the unexpected direction

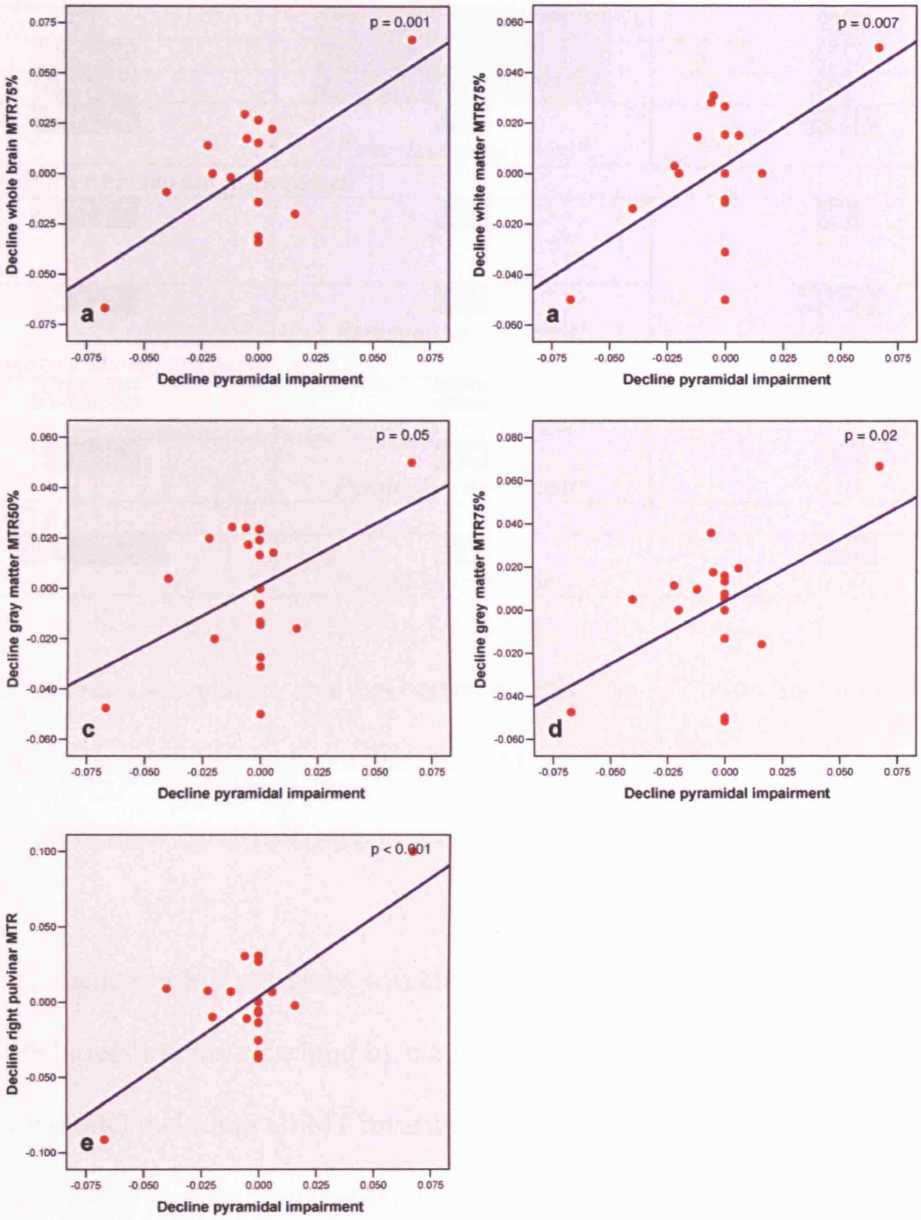


Figure 70: Graphs showing significant associations ($p<0.05$) between decline in MT measures and decline in pyramidal impairment (in the unexpected direction)

The above statistically significant ($p<0.05$) correlations are summarised in Table 25, with those MT measures highlighted which decline with worsening clinical scores, and unexpected associations indicated by an asterisk (*). Associations expressed in italics need to be interpreted with caution as they were possibly influenced by outliers.

Table 25: Summary associations between decline in MT measures and decline in clinical scores

Decline in slopes of MT measures	Decline in slopes of clinical scores	p value
a) Whole brain histogram parameters		

PH	*Extrapyramidal impairment*	*0.03*
MTR25%	*Extrapyramidal impairment*	*0.002*
MTR50%	*Extrapyramidal impairment*	*0.007*
MTR75%	CDR *Pyramidal impairment**	<0.001 *0.001*
b) White matter histogram parameters		
MTR50%	CDR GCS*	0.03 0.01
MTR75%	CDR *Pyramidal impairment**	p=0.002 *0.007*
c) Grey matter histogram parameters		
MTR50%	CDR *Pyramidal impairment**	0.02 *0.05*
MTR75%	CDR *Pyramidal impairment**	0.01 *0.02*
d) ROI MTRs		
Right pulvinar	CDR *Pyramidal impairment**	<0.001 *<0.001*

c) Spearman rank correlation analysis between decline in MT and clinical measures

All the above correlations between decline in MT and clinical measures were repeated with non-parametric Spearman rank correlation, and similar results were obtained.

d) Rate of decline in MT measures was also estimated for each patient with P102L and 6 OPRI mutation (as measured by the B value or slope) based on a linear regression model including all MT measures for that patient (outcome variable) and time (measured in months from baseline visit) as an explanatory variable. The statistical significance of this linear trend was assessed, but there was no evidence of a statistically significant ($p<0.05$) decline over time in any of the MT measures in any patient. In a similar manner, rate of decline in videoed and non-videoed clinical scores was assessed in P102L and 6 OPRI patients, but no statistically significant ($p<0.05$) decline was observed over time in any of the clinical scores in any patient.

Multiple significant ($p<0.05$) associations were observed when slopes of decline in MT measures were correlated with slopes of decline in clinical scores in P102L and 6 OPRI patients, and the statistical significance of their linear relationship assessed.

The slopes used to establish these associations were derived from the above analysis. Individual associations for P102L and 6 OPRI patients are described in Tables 26 and 27, though some associations were in the unexpected direction, indicated by an asterisk (*), and may be explained by the threshold effect (see Figure 71).

Table 26: Significant associations between decline in slopes of MTR histogram and ROI measures and clinical scores in P102L patients

Decline in slopes of MT measures	Decline in slopes of clinical scores	p value
a) Whole brain histogram parameters		
PH	Pyramidal impairment MMSE	0.03 <0.001
MTR50%	Extrapyramidal impairment	0.04
MTR75%	Cognitive impairment* Pyramidal impairment*	0.009 0.04
b) White matter histogram parameters		
PH	Cognitive impairment	0.02
MTR25%	MMSE	0.01
MTR75%	CDR	0.04
c) Grey matter histogram parameters		
AVMTR	ADAS-COG* Cognitive impairment* Extrapyramidal impairment*	0.04 0.006 0.04
PH	Pyramidal impairment	0.03
MTR25%	Cognitive impairment* Pyramidal impairment*	0.03 0.04
MTR50%	ADAS-COG* Cognitive impairment* Extrapyramidal impairment*	0.01 0.007 0.02
MTR75%	ADAS-COG* Cognitive impairment* Extrapyramidal impairment*	0.03 0.001 0.02
d) ROI MTRs		
R caudate	Extrapyramidal impairment* Cerebellar impairment	0.05 0.003
L caudate	ADAS-COG* CDR Cognitive impairment*	0.03 0.02 0.05
R putamen	ADL* ADAS-COG CDR*	0.05 0.02 0.01
L putamen	Cognitive impairment* Pyramidal impairment*	0.01 0.04
R pulvinar	Extrapyramidal impairment*	0.02
L pulvinar	ADL Cerebellar impairment	0.03 0.04

Table 27: Significant associations between decline in slopes of MTR histogram and ROI measures and clinical scores in 6 OPRI patients

Decline in slopes of MT measures	Decline in slopes of clinical scores	p value

a) Whole brain histogram parameters		
PH	Rankin*	0.03
MTR25%	Extrapyramidal impairment	0.04
MTR50%	CDR	0.02
b) White matter histogram parameters		
PH	Cerebellar impairment	0.03
PL	CDR	0.02
MTR75%	CDR BPRS	0.04 0.03
c) Grey matter histogram parameters		
PL	CGIS* Rankin* Pyramidal impairment	0.02 0.02 0.04
MTR75%	CDR	0.04
d) ROI MTRs		
R caudate	CDR CGIS* Pyramidal impairment	0.02 0.04 0.01
L caudate	MMSE* CDR* CGIS* Pyramidal impairment	0.03 0.01 0.04 0.02
R putamen	BPRS	0.02
R pulvinar	MMSE CDR	0.02 0.001

3.5.4.1 Missing data

Rating scales were only used at timepoints over the period during which MT measures were available, hence missing data from missed visits or failure to perform measures were few during these periods in a relatively well group of patients:

a) Baseline MMSE was missing in one patient with expressive dysphasia who had clinical assessments using the severely affected protocol (SAP).

b) Baseline ADAS-COG was missing in three patients, one assessed using the SAP and two others who declined.

c) Baseline GCS was not recorded in sixteen patients, usually by error of omission as these were not severely affected.

d) Baseline BPRS was missing in two patients, one patient was severely affected, and the other declined.

After baseline, analyses used observed data only.

3.5.4.2 Threshold effect (Figure 71):

Data from patients at different stages of disease severity was analysed at baseline and longitudinally. We would expect the greatest decline in MT measures in those with the greatest decline in clinical scores. However, a threshold effect can occur if those at an intermediate stage of the disease with the highest scores (e.g GCS 15) declined clinically at the fastest rates (e.g. to GCS 12) (slope A); whereas those at an advanced stage of the disease with the lowest scores (e.g. GCS 13) declined clinically at the slowest rates (e.g. to GCS 12) (slope B). If the decline in MT measures was greater in patients with poorest conditions, then paradoxically it is possible to get an unexpected result, with declining MT measures associated with better clinical scores; instead of deterioration. This is because the clinical score reaches a threshold after which it cannot deteriorate further.

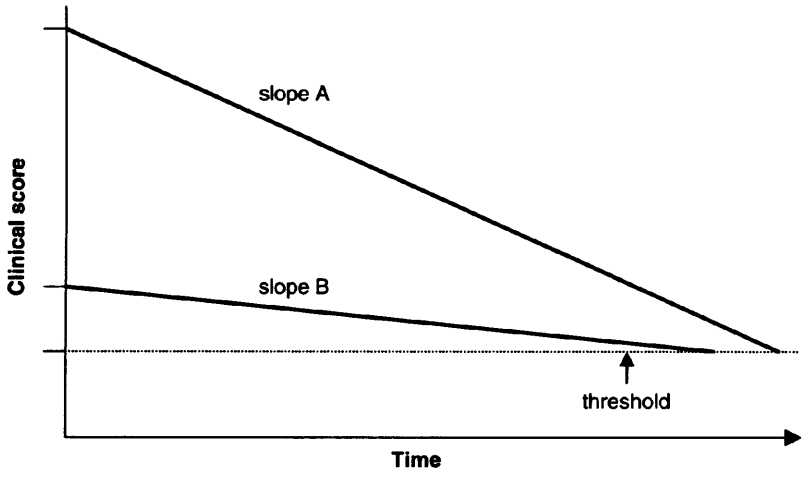


Figure 71: Diagrammatic representation of the decline in clinical scores leading to threshold effect. If MT measures decline more in patients with worst clinical condition at baseline paradoxically greater declines in clinical score can be associated with smaller declines in MT measures

4. DISCUSSION

4.1 SUMMARY OF FINDINGS IN THIS THESIS

4.1.1 Low MTR as a predictor of clinical disease severity

Lower MTR histogram parameters, particularly those obtained from whole brain and grey matter volumes were significantly associated with lower cognition, extrapyramidal impairment, cerebellar impairment, MMSE, CDR, ADAS-COG, CGIS and Rankin scores at baseline. Global MTR histogram measures were more robust than regional MTRs in predicting disease, as lower baseline mean ROI MTRs failed to show similar trends (except right putaminal mean MTR, which was associated with cerebellar impairment). This may well be because human prion disease causes diffuse disease affecting the cerebral and cerebellar cortices, basal ganglia and thalami [9]. Also, global MTR measurements in this study were more sensitive and robust than limited ROI measures used to identify localised pathology. A possible reason for difficulty in establishing significant associations between white matter MTR histogram parameters and measures of clinical disease severity is that grey matter is predominantly affected in prion disease with white matter involvement usually only seen in the panencephalopathic variant of sCJD [68]. None of the patients in this cohort had this unusual form of prion disease. Patients with human prion disease who took part in this study formed a heterogeous population of symptomatic and asymptomatic subjects with different PRNP mutations and at different stages in their disease course. This may also have affected results of the study and explain why MTR changes were observed more frequently in global than regional MTR measures. Other important factors relevant to this study which might have limited the identification of statistically significant regional MTR changes include the small

number of subjects involved, measurement errors associated with small ROIs and insufficient pathological involvement of the basal ganglia or thalami to produce detectable quantitative MT signal change. Analysis of conventional DWI/FLAIR images showed cortical signal change in only 1 patient (Patient 17) with a 6 OPRI mutation. Associations between MTR indices and clinical status has been observed at baseline in 22/26 patients in the absence of radiological abnormalities on conventional DWI/FLAIR sequences. Low global MTR values may therefore be a useful predictor of clinical disease onset at an early stage; thereby allowing earlier diagnosis and the possibility of entering therapeutic trials at the earliest possible clinical or pre-clinical disease stage. MTR measurement may also offer an objective and sensitive means of monitoring treatment response in such trials. A larger study is required to confirm these findings but data in this thesis demonstrates that MTR measures correlate with clinically meaningful measurements of disease severity and progression.

Across the study group as a whole there was no statistically significant longitudinal decline over time for any of the MT, and few of the clinical measures (except ADL, CDR, CGIS and Rankin), nor was there any association between decline in MT measures and baseline clinical scores, suggesting that baseline clinical scores were not independent predictors of subsequent decline in MT measures. Longitudinal decline in multiple whole brain, white matter and grey matter MTR histogram parameters and mean right pulvinar MTR correlated with longitudinal decline in extrapyramidal impairment and CDR. The paucity of associations on longitudinal analysis is explained by the fact that the median duration of illness in patients included in this study, the majority of whom have inherited prion disease, may be up to 7 years [44]. Most subjects were followed up at an early or intermediate stage of their disease, when they were able to undergo MRI scans and full clinical assessments and the

maximum duration of follow-up was not more than 29 months. Given the slowly progressive nature of their disease, the period of follow-up may not have been sufficiently long to detect longitudinal changes. Studies of patients over a longer period of time or with a more rapid disease course are needed to further establish the role of MTR measurement as a marker for disease progression and therapeutic intervention.

4.1.2 Low MTR as a biomarker of clinical disease

The hypothesis that MTR may provide biomarkers of clinical disease severity is supported by the finding that low MTR does indeed correlate with disease severity. The validity of this assumption was demonstrated in this study as several tests across a wide range of the clinical spectrum were significant independent predictors of low MTR at baseline. Baseline values of tests such as MMSE, Rankin, CGIS, cognitive, cerebellar and pyramidal impairment were predictors. Also, on longitudinal analysis MTR decline was associated with decline in CDR and extrapyramidal impairment. It is possible that patients have periods of greater MTR decline, usually at symptom onset (similar to the MRS changes described by Waldman et al [193]), at acceleration of symptom severity and when new areas of disability start; for example, when P102L patients with cerebellar symptoms start to develop cognitive disability late in their disease course. Alternative temporal patterns of MTR decline might be seen: for example patients whose clinical course 'accelerates' prior to death might have MTR decline at an accelerating rate; whereas those whose disease course has been slow but relentless may reach a 'plateau' phase where the rate of MTR decline is low and clinical function is severely affected but stable.

4.1.3 Best method for measuring change in MTR

Given that this study showed most associations between whole brain or grey matter histogram parameters and clinical measures at baseline, it seems appropriate to assess MTR changes using these parameters in the future for establishing early diagnosis as well as monitoring disease progression. As the underlying pathological processes may not affect the white matter, analysis of white matter histogram parameters may not be as useful as whole brain and grey matter histogram parameters.

4.1.4 Severely Affected Protocol (SAP)

The SAP was a novel technique developed to try and achieve a gradation of function within the severe spectrum of CJD. No existing scales for severe dementia adequately fit the clinical spectrum in CJD. The SAP did not prove to be a workable system for assessment of the severely affected CJD patients. Suggestions for an improved protocol in the light of the knowledge and experience gained from this study are presented later in this discussion.

4.2 FINDINGS IN CONTEXT OF OTHER WORK

4.2.1 Videoed examination

The use of the videoed examination to allow independent scoring, blinded to patient details, was a novel approach with much potential in prion disease. It is a technique originally pioneered for the movement disorders, particularly Parkinson's disease. Within prion disease, the use of video in the recording and assessment of Kuru in Papua New Guinea played an essential part in cataloguing the clinical features of this unique form of human prion disease and provided inspiration for the methods employed here [65]. Patients had consented to the use of their videos for research and teaching purposes so a useful library of signs has been collected over the time of the

PRION-1 trial which will help in educating and informing staff. Moreover, recording the clinical features of this disease visually will facilitate an understanding of the natural history of human prion diseases.

4.2.2 MTR measurements

This study used established acquisition and analysis techniques to measure MTRs in a population not previously investigated in this way. No specific modifications of these methods were required for this population with prion disease, other than a robust quality control process due to the common finding of movement artefact. To our knowledge there are no previous reports in the literature regarding MTR changes in prion disease.

To date in prion disease there has been no obvious candidate area designated as a disease-specific anatomical region of interest, unlike the hippocampus, amygdala and entorhinal cortex in AD[194-196], the caudate and putamen in HD [105, 197] and the superior cerebellar peduncle in PSP [198]. This is the first time that whole brain, white matter, grey matter, caudate nuclei, putamen and thalami have been segmented and characterised by quantitative MRI in prion disease.

Knowledge of the profile of MTR changes in prion disease, particularly at baseline, adds to the body of knowledge of MTR changes in dementia and contributes to an understanding of the factors affecting diagnostic sensitivity and specificity in this disorder. A recent cross-sectional study in AD [194], comparing global and regional MTR changes in whole brain and hippocampus, showed significantly reduced whole brain AVMTR ($p=0.002$), MTR25% ($p=0.03$) and mean hippocampal MTR ($p<0.001$) in patients as compared to controls; although unlike the present study no significant correlations could be established with MMSE. The particular challenge with prion

disease is recruiting sufficient numbers of patients into clinical trials to detect differences and patterns specific to different types of prion disease.

4.2.3 Identification of anatomical patterns of atrophy

Low MTRs observed in whole brain and grey matter volumes in this study may reflect a diffuse disease process and make recognition of localised pathology more difficult. Now that MTR changes have been identified and quantified in a heterogenous group of patients with prion disease the detailed neuropathological correlates of these changes should be established. For example, low MTR might reflect a global pathology affecting all glial cells in all brain areas, a cortical process in some patients, or a predominantly cerebellar process in others. In inherited prion disease different neuropathological processes and rates of disease progression are associated with different mutations, explaining why 6 OPRI patients had predominantly cognitive impairment and P102L patients had predominantly cerebellar impairment. Understanding the neuropathological basis of MRI changes, specifically the neuropathological changes that represent low MTR will be important. Studies are currently under way through the National Prion Clinic to compare neuropathological and MRI findings. Non-MT MRI sequences are also useful in identifying changes in the anatomical pattern of disease over time. For example, basal ganglia signal change on DWI seen in patients with sCJD may disappear late in the disease course [199, 200]. A combination of MRI sequences tailored to the type and clinical stage of prion disease may be required to provide optimal information.

Future studies will establish whether MT measurement can provide additional useful clinical information. This might be as a prognostic indicator in early prion disease or as a diagnostic marker in vCJD and sCJD. Prospective monitoring of asymptomatic at

risk individuals such as those exposed through potentially vCJD-infected blood transfusion may provide an opportunity to test these theories.

Obtaining scans in end stage disease is often impractical and may be inappropriate for severely ill, bedbound and mute patients. In both clinical practice and research consideration must be given to the ethical issue of consent, legal issues including the Mental Capacity Act (http://www.opsi.gov.uk/acts/acts2005/pdf/ukpga_20050009_en.pdf) and the balance of potential benefit versus risk for patients under investigation.

4.2.4 Outcome measures in clinical trials

One of the most difficult aspects to determine in a clinical drug trial is how to measure differences in outcome between groups. MT MRI has been used as a validated outcome measure for Alzheimer's disease in a therapeutic trial with donepezil, with responders having less severe structural damage to hippocampus and parahippocampus, as evidenced by less severe MTR changes compared to non-responders [201]. In terms of clinical outcome measures, for dementia as a whole, a set of validated tests have emerged such as the MMSE, ADAS-COG and Rankin scale that provide reliable and reproducible results. The challenge in this study was to find a set of tests which produced reliable outcome measures across the huge range of clinical severity and range of symptoms seen in prion disease. In some ways, the simplest tests bore out the best, with Rankin, MMSE and CGIS being useful predictors of change in MTR.

There are interesting correlates with other diseases such as Huntington disease (HD). A quote from a paper on outcome measures in HD by Wild and Tabrizi [202] holds very true for prion disease;

"HD progresses slowly and our current clinical assessments are inadequately sensitive to detect change reliably, even over several years and cannot distinguish between symptomatic benefit and disease modification. There are no established methods to assess disease progression in preclinical gene carriers. Therapies in mutation carriers early in the disease will probably have slight benefits and be associated with adverse effects. The pool of potential participants in HD is finite and trials using current outcome measures would require thousands of participants over long periods. These limitations might restrict the number of interventions that can be assessed, introduce potentially harmful therapies on inadequate grounds, or lead to decades of delay in making useful treatments available."

Trials such as PREDICT-HD [197] aiming to identify reliable markers of symptom onset in presymptomatic HD patients have parallels with the asymptomatic, mutation positive population with inherited prion disease. Preliminary results from this trial show that the volume of the caudate and putamen decreased significantly with an increasing likelihood that preclinical HD would become symptomatic; cognitive, motor and neuropsychiatric scores were also significantly predictive.

4.2.5 Statistical models

Linear regression models have been used to analyse results from longitudinal clinical trials and observational trials, for example in HD [203], where a very similar approach to ours was used, with baseline predictors of rate of decline being investigated in addition to rates of decline in clinical scores. Similarly, baseline Spearman rank correlation analysis has been carried out in HD [105], correlating MT, DWI and volumetric MR changes with functional disease capacity and severity.

4.3 ADVANTAGES AND LIMITATIONS OF THIS STUDY

4.3.1 Advantages

The reproducibility of high quality MRI data from this unique patient cohort at The National Hospital was of note. It was the first investigation of MT imaging in human prion disease, as part of the first therapeutic trial of quinacrine in CJD in the UK, with contributions from a multidisciplinary team of researchers comprising clinicians, neuroradiologists, physicists and specialist nurses.

Although some scans had to be excluded due to motion artefact or patient non-compliance, there were very few machine-related technical reasons to exclude scans. A factor that improved inclusion rates considerably was the introduction of performing MRI under general anaesthetic. Patients whose previous scans had been excluded due to motion artefact provided good quality MT data sets and patients with claustrophobia or severe myoclonus were able to tolerate the scanning procedure. One strength of this study was that 1 investigator (myself) was responsible for all MRI data analysis. Inter-rater variability was therefore not a confounding factor in calculating MTRs, ROI selection or segmentation.

The technique for acquisition of the video assessments was highly developed. Videoed and non-videoed examinations were all performed by 2 experienced registrars (myself and a trial fellow), who learnt the PRION-1 protocols during the pilot phase. They followed up their own cohort of patients, optimising accuracy of longitudinal clinical assessment. The specialist nurses who manned the video cameras were likewise experienced in the practical videoing of the examinations, adhering to the storyboards to achieve optimal images and sound.

Statistically, the use of linear regression analysis implied that missing data values did not have to be estimated using methods such as 'last observation carried forward' or

'missed=fail'. These methods introduce their own types of bias; for example, deceased patients keep scoring at the level of their last assessment throughout the trial. This was also considered particularly important since the reasons for tests being missed were wide and varied. Simple estimations of missing values might not have represented the underlying reasons for absent data.

4.3.2 Limitations

4.3.2.1 Study population

In the analysis described in this thesis all patients with prion disease were considered together for the purpose of relating MTR changes and clinical measures, although most of the patients were symptomatic inherited patients. The findings of the study may therefore be most applicable to symptomatic inherited patients, as considerably less information is available for variant, sporadic and asymptomatic inherited patients.

4.3.2.2 Controls

The power of this study would have been increased if an age and sex matched control group had been available, recruited at the same time as the PRION-1 patients and scanned at similar intervals. In order to control for environmental factors, non-affected siblings or close family members could have been used. The need for this group was not anticipated when the PRION-1 trial was originally planned. The National Prion Monitoring Cohort Study has provision to study control subjects recruited from asymptomatic mutation-positive family members.

4.3.2.3 Spacing of scans

A balance has to be made between inter-scan intervals that are too long to catch longitudinal MTR decline in rapidly progressing disease or asymptomatic to symptomatic switching and intervals that are too short to detect change in stable or asymptomatic disease. Provision was made for this to a certain extent in PRION-1 as asymptomatic patients were scanned annually, all other patients being scanned at intervals according to the PRION-1 protocol.

The cohort of patients with prion disease investigated in this thesis often had maximum scan intervals of less than 6 months. Due to the small number of patients it was felt better to analyse MTRs in all patients regardless of maximum scan interval. The limitations of shorter scan intervals had to be accepted, because shorter scan intervals in inherited patients (who tend to have prolonged duration of illnesses) would possibly be unable to detect any appreciable change in MTR, as these patients do not clinically deteriorate as rapidly as variant and sporadic patients. An advantage for this study of the shortest scan interval of 0, 4 and 8 weeks was the inclusion of one sporadic, one variant, one P102L, 2 6 OPRI and 1 A117V patient, who were either only assessed at baseline or whose longest interval was shorter than 8 weeks. These patients would otherwise have been excluded from analysis.

Problems with annual scanning of asymptomatic patients included technical problems with the scan (such as the patient moving), which meant that an interval of more than one year sometimes occurred. There was then the potential that no data was acquired just before the onset of clinical symptoms. As the MRI scanner can be a claustrophobic and is a noisy environment, stable or asymptomatic patients would sometimes elect not to have an MRI, and 'wait until the next visit', with the same potential problem. Sometimes patients who were worried that symptoms might be starting would elect not to have an MRI for fear of symptomatic disease stage onset

being confirmed. If the index of worry for disease onset becomes higher during a routine visit, scans would be better planned more frequently, at least 6 monthly.

4.3.2.4 Quality control process

Twenty-seven out of 115 MT scans were excluded during the quality control process. This large loss of data in the investigated patients with prion disease was due to unavoidable movement artefact compared with that expected from the healthy population. MTR measurements are very sensitive to motion and cannot be made accurately when movement exists. In order to minimize variability within the data, as required to sensitively detect the expected small changes in the MTR indices in this population, only perfect or near perfect MT data sets could be included in the final analyses. An unfortunate effect of the quality control process was that 3/4 variant patients were completely excluded; it was considered essential to apply consistent inclusion criteria to all data sets acquired in the trial rather than making exceptions (and therefore increasing error) for certain disease types.

4.3.2.5 MTR measurements in ROIs, whole brain, white matter and grey matter

In order to more fully characterise measurement reproducibility, repeated MTR measurements in ROIs, whole brain, white matter and grey matter could have been undertaken by the same researcher, or by other researchers, and the inter- and intra-rater reproducibility calculated. Any learning effects with experience or systematic errors in analysis could have been identified.

During initial ROI analysis, attempts were made to calculate MTRs in frontal, temporal, parietal and occipital cortices, but accurate values could not be recorded as

it was difficult to delineate these areas without including the white matter. This technique could be improved in the future.

Grey matter MTR histograms were generated from tissue volumes obtained using a maximum likelihood segmentation of the grey matter using validated and widely-used software. Although this volume did comprise mostly of the caudate nuclei and cortical grey matter, the putamen and thalami could not be segmented, which is a common problem of segmentation using this approach. Preliminary attempts were made to overcome this, but time constraints prevented substantial improvement: future work exploiting recently available, more sophisticated segmentation algorithms (e.g. those implemented in SPM5 or SPM7) should allow the proper segmentation of these anatomical structures.

4.3.2.6 Videoed and non-videoed clinical examination

Numerous clinical scores were recorded with or without video, with a lot of overlap and redundancy, especially on videoed examination, but it was important to assess which tests worked best in prion disease. Carrying out clinical assessments was a time consuming and laborious task, not only for cognitively impaired patients, but for the researcher too, but a useful one to formulate a standardised assessment by the independent neurologist for videoed examination and subsequently produce a standardised examination in prion disease based on both videoed and non-videoed clinical scores. Though sometimes there were verbal clues to diagnosis on videos, they were dubbed, or edited out, as much as possible to keep the independent neurologist blinded to diagnosis.

The obvious visual sign of quinacrine usage (quinacrine imparts a lemon yellow tint to the skin) was obliterated with the use of a yellow filter over the DVD player. Other

visual clues to diagnosis were impossible to remove. It is not hard to guess that a small cohort of teenage patients might have variant disease, a cohort of middle-aged patients might have inherited disease and an older population, bedbound, mute with myoclonus might have sporadic disease. These patient stereotypes do not always hold true however, and it is equally important to blind to the chronological sequence of follow up visits for this study.

A common reason for data to be missing was that the independent neurologist could not hear or interpret the response. As far as possible the recording of sound and video was optimised with good quality digital video recorders and directional microphones on both the table and tripod cameras. Despite this, the dysphasia in many patients meant that tests could not be scored; equally other causes such as loud background noise, loss of sound on the video and extremely quiet speech had the same score outcome.

4.3.2.7 Statistical analysis

There are two main areas of difficulty in the statistical analysis in this thesis. The first is that relatively small numbers of patients were involved and often a non-homogenous clinical phenotype was seen even within the same diagnostic category (particularly in sporadic cases). It was anticipated at study onset that a larger number of scans than that available for the final analysis would be obtained, given the fact that most patients were being scanned at regular 3-month intervals. However, this failed to be the case in this progressive disease for various reasons: many scans were excluded due to motion artefact, intervals between scans were unavoidably prolonged to more than 3 months, and it was difficult to obtain consent for scans under GA. Secondly, an

assumption of a linear relationship between the various test metrics was made, regardless of scoring structure or tendency to categorical values for scoring.

Multiple statistical comparisons have been performed in this study. The interpretation of p values or any corrections used must be considered in the light of this multiple testing. There are two aspects to consider. Firstly when multiple explanatory variables (such as different cognitive tests) are assessed on a single outcome (e.g. AVMTR in whole brain) there is a problem of confounding. The best way to correct for this is to fit a multivariate regression with variable selection. The second aspect is where multiple outcomes are considered e.g. change in MTR in many regions (ROIs, whole brain, white matter and grey matter). Techniques such as Bonferroni and Simes corrections can be used where multiple outcomes are considered. It is possible to adjust the p values to allow for multiple testing with these tests. Bonferroni considers a result statistically significant at the 5% level if $p<0.05$/number of tests. Simes orders the p values, then accepts as significant at the 5% level the first test if $p<0.05$/number of tests, the second test if $p<0.05$/ (number of tests-1), the third if $p<0.05$/ (number of tests-2) etc. The p values in this study have not been adjusted as it is probably more important to be able to understand what the p value means in the context of each test, rather than trying to adjust the level at which significant differences are achieved. However, for baseline Spearman rank correlation analysis, a lower significance level of $p<0.01$ has been used to ensure adjustment for multiple testing.

The majority of the statistical tests used in this study were affected more by confounding than multiple outcomes. A more important problem is the small numbers of patients involved. P values will not be large and power is extremely low. The ability to identify real effects is simply very small and there is no way to improve this without larger numbers. The small numbers involved meant that the overall changes

in MTR observed in the regression analyses were largely dominated by those subjects with the greatest number of scans; i.e., the symptomatic inherited prion disease patients. While this group did comprise the majority disease type within the trial patients, the analysis employed may have further biased the results towards their influence. There were also variable time intervals between scans, different number of scans for different patients, or fewer scans obtained over a shorter duration of follow-up. The only way to correct for these factors is for patients:

a) to have the same time interval between scans and same number of scans, or

b) to have as many scans over as long a time period as possible.

All analyses also assumed that the baseline scan was the 'gold standard', measured without error.

The linear regression model is best used in a homogenous group; however this was not the case in the population assessed here. Large changes in MTR over short periods of time were expected to be accurate in sporadic or variant disease, rather than representing a measurement with a lot of error due to the short time intervals involved. This method did not give as much weight to those patients declining faster over the shortest intervals. The only way to give these subjects' change in MTR more appropriate weight would be to have multiple scans over shorter intervals.

4.3.2.8 Statistical methods for dealing with missing data

Linear regressions were used since reasons for missing data were varied (as discussed previously under section 3.3). All analyses of slopes made a 'missing completely at random' assumption, i.e. that missing values did not depend on any important factor. This is questionable as sick patients were likely to be too unwell for examination; for

example in the SAP, so this data was not 'missing not at random'. The use of linear regression was considered the best approach to deal with these problems.

GCS measurements were often not recorded as the patients were able to perform the MMSE instead. However, determination of the time when GCS became less than 15 was lost. Conversely, during videoed neurological examination, almost all the walking scores were missing due to the patient being unable to stand, and data was missing as there were inadequate means of recording this on the trial forms.

Overall it was felt better not to try and estimate missing values directly due to the range of reasons for their absence, as well as the assumption that linear regression would allow for the missing values.

Disadvantages of the linear regression method included that periods of rapid change may have been missed due to the effective averaging out of points over the timescale of the trial. Also, estimations of rates of change generated over very short intervals were more susceptible to error and, again, may not have been representative of the pattern of change over the time course of the trial.

Some analyses regressed estimated quantities on other estimated quantities (e.g. decline in clinical score against decline in MTR). Usually this method regresses observed factors on other observed factors which are assumed to be without error. Within a model, an error for the dependent variable is fitted; the fact that the independent variables here were measured with error is a potential weakness.

4.3.2.9 Quinacrine

The small numbers of patients and the 'patient preference' aspect of the PRION-1 trial caused large problems in interpretation of the clinical trial analysis and

fundamentally questioned whether analyses of an effect of quinacrine were meaningful to perform in the first place.

The PRION-1 trial was powered to detect statistically significant differences between groups but assumed that the majority of patients would choose randomisation, which they did not. Any statistically significant difference found between treated and non-treated patient groups might reflect selection bias for each study population.

Many factors could affect the patient's decision of whether or not to choose quinacrine. These factors would also have to be involved in matching patients. Some factors can be measured within the trial, such as severity of disease, but other potentially significant factors (such as presence of an enthusiastic relative, recent media coverage or provision of sufficient social care) need to be taken into account and cannot or have not been formally measured.

The length of time that quinacrine was taken and the dosage varied sufficiently such that the 'quinacrine' group were heterogeneous, even before association with MRI and clinical measures were made. Ideally the same dose should have been used for the same length of time in all patients. Reasons for changing dose or stopping quinacrine were varied but mostly independent of disease stage (e.g. liver toxicity, nausea and rash rather than inability to swallow or decreased level of consciousness), thus no useful information on disease stage and stopping quinacrine can be made.

If there were large numbers in both quinacrine and no quinacrine groups, and they were well matched then interaction effects could be better ascertained. Possible interaction effects could be qualitative (e.g. association absent on quinacrine, association present off quinacrine) or quantitative (e.g. association smaller on quinacrine than off quinacrine). Most potential interactions in the context of MRI and clinical measures would be quantitative and many different kinds of analysis could

have been performed comparing quinacrine with no quinacrine groups. However, any associations found would be uninterpretable, because any effect seen might be due to differences in the kind of patient who chooses quinacrine or not, (which cannot be adjusted for), and not due to quinacrine itself. Similarly, even if no associations are found, there might be a large quinacrine effect obscured by lack of comparability which again cannot be adjusted for.

As quinacrine is a stimulant, it might be predicted that clinical measures reflecting increased levels of attention (such as words beginning with the letter F, A or S on videoed neurological examination) would not decline, or would improve. An obstacle in assessing this is that the baseline clinical assessment and scan were often done soon before the quinacrine was started which did not allow sufficient time to properly assess level of functioning before the treatment was commenced. Ideally at least two measures could have been performed before quinacrine was started with as long an interval as possible.

Ideally clinical data used to inform decisions on disease specific clinical severity scores should have come exclusively from patients not taking quinacrine or off quinacrine for a suitable length of time. However, for a small treatment group where, at best, only short term improvement or slowed disease progression was anticipated it was felt reasonable to include all patients.

4.3.2.10 General anaesthesia

Careful examination of the data suggested a trend for histogram PH to be slightly higher in some patients when they had serial scans under GA compared to those serial scans which they had without GA. As most scans under GA were done when patients were at an intermediate or an advanced stage of their disease, it is difficult to assess

whether this effect may be due to underlying disease, effects of GA upon cerebral MTR values or more likely due to reduced subtle motion artefact. Further investigations with a larger patient group are needed before any definite conclusions can be made regarding this possible effect.

4.4 POTENTIAL FUTURE WORK - MRI

Ideas for improvements to any future clinical trials in prion disease are proposed, as well as severity scales in prion disease in light of the results produced.

4.4.1 MRI as an objective measure in future trials

Clinical assessment in prion disease is challenging due to the diverse range of symptoms and disease severity. Many of the cognitive and motor measures used in this thesis are affected by variable factors such as mood, intellectual ability, motivation or fatigue as well as neurological dysfunction such as apraxia or dysarthria. MTR is an objective measure unaffected by such factors and unable to be altered intentionally by the patient. It is a tool that can be used across all types of prion disease at all stages of disease and is therefore potentially one of the most useful independent measures of disease progression in future clinical trials in prion disease.

4.4.2 Proposals for scan intervals and duration in future clinical trials in prion disease

Asymptomatic patients on yearly scanning should be scanned 6 monthly. Variant and sporadic patients should be scanned every 4 weeks if they are able to tolerate it. Symptomatic inherited patients should be scanned at 3 monthly intervals, and GA should be utilised with valid consent particularly in patients whose scans are degraded

by severe motion artefact. Careful trial protocol planning is required to optimise the number of scans performed in each disease type to sufficiently power the study. The total MT scan duration was 12 minutes, which makes it difficult for cognitively impaired, myoclonic patients to lie still. Motion artefact could be reduced by developing improved acquisition techniques which may reduce the scanning time.

4.4.3 MTR measurements in ROIs, whole brain, white matter and grey matter

The future for determining MTRs in whole brain, white matter and grey matter undoubtedly involves automated techniques, rather than time consuming manual definition of ROIs. Additionally, development of automated techniques to delineate cortical and cerebellar grey matter would be very useful in prion disease, involving predominantly the grey matter. This would also be helpful in delineating basal ganglia and thalami without inclusion of CSF. Methods for automated calculation of whole brain, white matter and grey matter MTR histogram parameters need to be optimised for inclusion of respective areas only, without contribution from surrounding brain tissue or CSF. Automated analysis of cerebellar MTR histogram parameters would be particularly useful in monitoring patients with InhPrD; for example, P102L mutation, where ataxia precedes impaired cognition due to onset of cerebellar atrophy.

4.4.4 Future use of imaging in diagnosis of prion disease

A future area of study is the labelling of PrP using a marker visible on an imaging technique (for example PET) which would be specific to prion disease and which would form the basis of a diagnostic test in vCJD and sCJD, without the need for biopsy.

4.4.5 Future applications of neuroimaging

MT and non-MT MRI both have roles in monitoring progression of prion disease but may be stronger in combination than alone. Future research will be directed towards finding the earliest point in the disease course where imaging changes are evident. Additional quantitative techniques such as DWI, diffusion tensor imaging and volumetric imaging may be of use firstly in pre-symptomatic patients (either inherited or exposed to infectious prion protein) to help predict the onset of clinical disease and, secondly, in symptomatic patients measuring the response to treatment in therapeutic trials. As current and future MRI sequences evolve, those showing the earliest changes, with the highest sensitivity and specificity should be prioritised in diagnostic imaging.

4.5 POTENTIAL FUTURE WORK – CLINICAL ASSESSMENTS

Part of the reason for assessing predictors of MTR change was to provide evidence for those tests which will be most useful in monitoring disease progression in prion disease. The group of tests used was intentionally large; there was significant overlap and thus redundancy in the testing. Prioritising tests that approximately mirror change in MTR will make clinical assessment more efficient and reproducible in the future.

4.5.1 Which non-videoed tests should be used?

The non-videoed assessments such as MMSE, ADAS-COG, Rankin, Barthel ADL, CDR and CGIS were applicable and useful in all patients with prion disease. MMSE and ADAS-COG were the 2 most comprehensive cognitive tests. The decision regarding which test to use should be tailored to symptom state. The ADAS-COG was best used when trying to identify the point at which a patient changed from an

asymptomatic to symptomatic state and MMSE was a better general measure. Repeated measures of both tests are important to allow decline to be assessed. Baseline MMSE was also a significant predictor of change in MTR, though ADAS-COG was not. P102L patients had higher scores in both MMSE and ADAS-COG for longer than 6 OPRI patients as they manifested their decline more in cerebellar function and mobility than cognitive function.

Activities of daily life were measured with the more complex Barthel ADL and simpler Rankin. Barthel ADL was superior due to greater range of activities considered and greater range of outcome scores. The range of scores in Rankin was low and many patients did not change scores throughout. This was not necessarily a criticism as most inherited symptomatic patients were stable clinically and Barthel ADL scores could fluctuate more in these patients. However, Rankin score was a significant predictor of MTR change at baseline, while this was not observed with Barthel ADL score. Rankin is a quick and easy test to perform and there are few grounds for excluding this test from future clinical trials. The more detailed assessment used in the Barthel ADL index could be more useful in the least affected or asymptomatic patients.

CDR scores subtly different functions to the tests of activities of daily living above and is a useful comparison as it may detect differences more applicable to patients with prion disease or a subgroup of it. Decline in CDR was significantly associated with decline in MTR. Being sufficiently different, but just as useful, it should be included in future clinical trials.

CGIS is the most subjective of the tests, as it is dependent on the rater's opinion which may be affected by knowledge of disease type and length of symptoms. However, it is also free of the restraints of fixed determined outcome scores that other

tests have. If the other tests are too rigid to detect or measure change in patient status this may be the only way of getting a true global picture of decline. Separate doctor and nurse CGIS were scored in the trial, though only the scores recorded by doctors were used in this thesis; in future a consensus view should be sought and recorded. Baseline CGIS was a significant predictor of change in MTR. That a subjective clinician's assessment was as good a measure as objective tests may reflect the clinical experience of prion disease that the PRION-1 clinicians acquired over the course of the study. With less experienced or a greater number of clinicians assessing patients there might have been more variability in scores, thus making CGIS a less significant predictor. Trial clinicians carried individual caseloads so they followed up their own sets of patients, and were thus in the best position to identify change. The theory behind the CGIS score is that it is an instrument which measures clinically meaningful change, rather than a measure of the score's use in assessing change. CGIS is not intended to be a sensitive measure of small changes that are unlikely to be clinically meaningful. In principle, a clinician rating a subject as changed on a global change scale is determining clinically meaningful and distinct change. Therefore, any change recorded on a CGIS is considered clinically significant by definition.

CGIS should be included in future clinical trials as it is a simple and quick test, but a small number of experienced clinicians should do the rating and each patient should be followed up by the same clinician. If time is limited then either the CGIS or the Rankin should be omitted as both have very similar scales and are equally good predictors of change in MTR.

GCS is a very useful tool in assessment across all types of prion disease. Its utility was compromised by a high rate of missing values due to poor trial design. This test

should certainly be performed and due to its simplicity could be reliably completed in all patients at all stages of disease (an uncommon virtue amongst tests).

A summary table of these findings is shown below:

Table 28 Which non-videoed tests should be used in a future trial for prion disease?

TESTS TO KEEP	AREA OF MOST USE
MMSE Rankin GCS	Global
ADAS-COG Barthel ADL	Early symptomatic disease
CGIS MMSE Rankin	Significant predictors of change in MTR
GCS	Terminal decline

The global tests and significant predictors of change in MTR at baseline, as well as longitudinally, should be used in all patients and tests best suited to early disease or terminal decline utilised in the appropriate patients.

4.5.1 Which videoed tests should be used?

Lower MTR histogram parameters, particularly whole brain and grey matter histogram parameters, were significantly associated with lower cognition, extrapyramidal impairment and cerebellar impairment. Cognitive, cerebellar and pyramidal impairment were independent predictors of low MTRs at baseline and MTR decline was associated with decline in extrapyramidal impairment. Thus, it is of paramount importance that cognitive, extrapyramidal, pyramidal and cerebellar impairment is videoed and the scores independently recorded, for assessment of disease progression and monitoring response to treatment in future therapeutic trials. The drawback is that sometimes it may be difficult to assess extrapyramidal or pyramidal impairment on video, particularly in the early stages of the disease when

there is only mild impairment and verbal clues need to be provided to the independent assessor.

Videoed scores provide a comprehensive assessment of a patient's clinical condition in all forms of symptomatic and asymptomatic prion disease. Even though BPRS did not reveal any significant associations with low MTRs (as clinically there was not a marked change in patients, nor were independently assessed scores used in this thesis), it will be useful in the future to assess the psychiatric status of the patient independently on video as this is a clinically important area for patients and their carers.

4.5.3 Suggested future use of rating scales in severely affected patients

The Severely Affected Protocol was an unsatisfactory method for measuring function and disability in the PRION-1 trial. Further thought on how to assess these patients is required, particularly as increasingly patients with prion disease are surviving for months and sometimes years in end stage disease. This is particularly true of variant prion disease; improved nursing, nutrition via gastrostomy tube and high vigilance for infection mean the average life expectancy in this young group of patients is probably higher than the quoted 14 months in the literature.

There is a need for a comprehensive scale that can be applied to all patients, but which does not have the 'flooring' problems of losing the ability to differentiate between the final few levels of function. Delineation of these symptoms is important, particularly in trials in prion disease, where the aim to date has been to halt progression of disease, rather than make improvement. If the level of severe impairment at baseline cannot be adequately measured, there is no hope of detecting a treatment effect.

A potential comprehensive rating scale is the Disability Rating Scale (DRS) which was designed to assess improvement in moderate and severe patients with traumatic brain injury. It is mixture of the Barthel measure of ADL and GCS with additions. Areas covered include: Eye opening, Communication ability, Motor response, Feeding, Toileting, Grooming, Level of functioning and Employability. The DRS is however not particularly sensitive at the lower end of the scale, so although an improvement on the current severely affected protocol, additional tests to delineate these patients would be desirable (see Coma/Near Coma Score below).

Disadvantages of comprehensive scales such as this are also that the subtle early signs in previously asymptomatic patients are often not detected. A solution might be to incorporate aspects of different scales, such that detection of change is focussed on areas significant in the patient's type and stage of prion disease; for example doing a full ADAS-COG and MMSE on all asymptomatic patients at each visit.

Another comprehensive rating scale is the Total Functional Capacity score (TFC) which is used to grade progression of symptoms in HD [204] (Table 29).

Table 29 Total Functional Capacity Scale

Total Functional Capacity (TFC) Scale					
	Engagement in occupation	**Capacity to handle financial affairs**	**Capacity to manage domestic responsibilities**	**Capacity to perform activities of daily living**	**Care can be provided at...**
Stage I (TFC 11-13) (0-8 years)	Usual level	Full	Full	Full	Home
Stage II (TFC 7-10) (3-13 y)	Lower level	Requires slight assistance	Full	Full	Home
Stage III (TFC 3-6) (5-16 y)	Marginal	Requires major assistance	Impaired	Mildly impaired	Home
Stage IV (TFC 1-2) (9-21 y)	Unable	Unable	Unable	Moderately impaired	Home or extended care facility
Stage V (TFC 0) (11-26 y)	Unable	Unable	Unable	Severely impaired	Total care facility only

This scale details level of function in the domains of workplace, finances, domestic chores, activities of daily living and requirements for unskilled or skilled care. It has the benefit of being in regular use and validated in HD, a disease with a similar profile of asymptomatic through to severe disease. The additional measurement of where care is provided is a useful measure not covered in other scores. In prion disease access to care at home or hospice is available to all due to funding from the National Care Package, so is not dependent on income.

Several scales have been developed for the severely affected group of patients with dementia. The Level of Cognitive Functioning Scale (LCFS) has potential use as it covers the spectrum from normal to severely affected but better delineates the later stages. It is an eight point scale gradating the presence and appropriateness of response to general external stimuli. It is effectively an extension of the GCS, but more tailored to a declining patient with dementia:

1-No response

2-Generalised response

3-Localised response

4-Confused, agitated response

5-Confused, inappropriate, non-agitated response

6-Confused, appropriate response

7-Automatic, appropriate response

8-Purposeful, appropriate response

This would be a useful scale to use in all variant and sporadic patients from the first assessment, in order to catch the point at which less than full scores are obtained and to provide an overall measure of decline. An indication of appropriate score could be

gained from carers and relatives, particularly if there is fluctuation in severity. One of the drawbacks of the PRION-1 assessments was that a brief snapshot of function was gained and rated, which may not have been representative of current level of functioning. Carers' assessments were not always possible. The LCFS should be piloted before future trials to ascertain its effectiveness as it seems a useful and practical test in end stage prion disease.

The Severe Impairment Battery (SIB) is another alternative to the SAP, however it has too many shortfalls that are similar to the SAP such as 'flooring' of scores, so would perhaps add little.

Two potential tests in pre-terminal stages are the Coma Recovery Scale (CRS) and the Coma/Near-Coma (CNC) Scores. Pre-terminal patients in PRION-1 who had few clinical assessments and videos (and usually no MRIs) often had abnormal patterns of respiration or were mute and unresponsive. Current assessment did not include measurement of pre-terminal markers, yet progression in these patients was often visible on follow up assessments. The Coma Recovery Scale was initially described by Giacino in 1991 [205] and revised in 2004 [206]. It was designed to assess patients with disorders of consciousness. The scale has 23 items in six subscales covering auditory, visual, motor, oromotor, communication and arousal functions. The subscales are organised into hierarchical brain stem, subcortical and cortical processes. The lowest score on each subscale represents reflex activity while the highest scores represent cognitively mediated behaviours.

The CNC [207] expands the levels of the Disability Rating Scale (DRS) to include the vegetative categories. It was developed to measure small clinical changes in patients with severe brain injuries. The CNC has 5 levels, based on 11 items that can be scored to indicate severity of sensory, perceptual, and primitive response deficits.

The 11 items are:

Auditory: Bell ringing

Command Responsivity: Request patient to open or close eyes

Visual: Light flashes

Visual: Tracking

Threat: Eye blink to threat

Olfactory: Ammonia capsule

Tactile: Shoulder tap

Tactile: Nasal swab

Pain: Firm pinch on finger tip

Pain: Robust ear pinch/pull x3

Vocalisation: Best response.

The total score is calculated as a mean response from the 11 items. Use of the DRS combined with the CNC for patients in the lowest band of scores for the DRS should be piloted. This would extend the spectrum of disease that could be measured to include more severely affected patients. Due to its all encompassing nature, it may obviate the need for the other separate scores.

There are many scales that could be used in patients with prion disease that have been used in other dementias. Revisiting which scales should be used in which patients, or alternatively choosing comprehensive scales to apply to all patients at all stages of disease would be a useful exercise, as there are several easily applicable alternatives.

4.6 HOW SHOULD DISEASE SEVERITY IN PRION DISEASE BE MEASURED?

PRION-1 used a wide range of clinical tests on a population with prion disease to assess which tests best represented decline or change. There is currently no classification of disease severity in prion disease, similar to that obtaining in Alzheimer's disease [208]. AD is a better studied disease with more patients in whom markers of severity can be assessed and validated. Development of a 'Global Severity Score in prion disease' comprising various aspects of clinical tests, that could be tailored to the clinical scenario, would be advantageous as a clinical tool. Markers of change between asymptomatic and symptomatic disease would be useful both for inherited patients, as well as the cohort of patients exposed to vCJD via infected blood transfusion. These markers would be useful in the future for monitoring other at risk individuals exposed to infectious prions through an iatrogenic medium such as blood transfusion, infected surgical instruments or endoscopes. A sensitive and specific marker is required urgently for these circumstances. Future cohorts of at-risk patients from these groups might be used to validate such a marker. No asymptomatic patients became symptomatic during the course of this study. A group of 6 asymptomatic patients are being followed up (and will continue to be followed up after PRION-1 within the auspices of the National Prion Monitoring Cohort). Experience gained from when these 2 P102L, 1 E200K, 1 A117V, 1 D178N and 1 5 OPRI patients become symptomatic will be invaluable in informing clinicians of the pattern of change. Unfortunately there are currently no 6 OPRI asymptomatic patients being followed up.

Baseline values of tests such as MMSE, Rankin, CDR, ADAS-COG, CGIS, cognitive, cerebellar, extrapyramidal and pyramidal impairment mirrored change in MTR most closely. Also, MTR decline was associated with decline in CDR and extrapyramidal

impairment. Using the information gained in PRION-1 trial the following clinical severity markers are suggested in different disease types:

P102L:

Typical disease course includes early cerebellar signs followed by loss of lower limb reflexes and confinement to wheelchair, with later cognitive decline and reduced GCS.

Stage 0: Asymptomatic, with normal cerebellar and cognitive function.

Stage 1: Presence of one or more cerebellar signs on consecutive assessments (finger-nose testing, wide-based gait, heel-shin test, nystagmus on eye movements), or not normal on videoed cerebellar impairment score

Stage 2: Loss of lower limb reflexes

Stage 3: Confined to wheelchair for most daily tasks

Stage 4: MMSE <28 or ADAS-COG >10

Stage 5: GCS <10

6-OPRI:

Typical disease course includes early cognitive decline, demonstrated first on ADAS-COG, with increasing severity of cognitive symptoms, such that Barthel ADL score falls below half, MMSE falls to a third and finally GCS drops.

Stage 0: Asymptomatic, normal cognitive function

Stage 1: Score of >5 on the ADAS-COG on consecutive assessments

Stage 2: Score of >10 on the ADAS-COG on consecutive assessments

Stage 3: Barthel ADL <10

Stage 4: MMSE <10

Stage 5: GCS <10

Sporadic:

A simpler scale is required in sporadic patients due to late presentation to specialist services and rapid progression of symptoms

Stage 0: Asymptomatic, normal cognitive, cerebellar and psychiatric features

Stage 1: Able to attempt all questions in MMSE

Stage 2: Barthel ADL <10

Stage 3: GCS <10

Stage 4: Bedbound and mute

Variant:

The need to identify symptomatic disease exists due to the cohort of infected blood recipients.

Stage 0: Asymptomatic, normal cognitive, cerebellar and psychiatric features

Stage 1: Mild social withdrawal, depression, uncharacteristic behaviour

Stage 2: MMSE < 28 or ADAS-COG > 10

Stage 3: Barthel ADL < 10 or MMSE <10

Stage 4: GCS <10

Stage 5: Bedbound and mute

Overall:

An overall scale that may be used across all types of prion disease is described below:

Stage 0: Asymptomatic, normal cognitive, cerebellar and psychiatric features

Stage 1: Score of 5 or more on ADAS-COG or cerebellar symptoms

Stage 2: Barthel ADL <10

Stage 3: GCS <10

Stage 4: Bedbound and mute

In future trials MTR decline could be correlated with stage of disease to assess the prognostic use of MTR decline. Finding the right combination of markers of disease

severity in each disease type will make disease staging and therefore prognostic information more robust. It will also allow easier assessment of change in therapeutic trials if disease severity has been delineated.

4.7 FUTURE TRIAL DESIGN

Patient recruitment is crucial for achieving sufficient power in comparisons between groups. Matching or balancing numbers of controls, of both asymptomatic and symptomatic patients, is important. Ideally, an equal number of symptomatic patients should be compared to an equal number of matched controls and similar comparisons should be made between asymptomatic patients and controls. Asymptomatic patients may have an acceleration in MTR decline prior to symptom development, similar to the MRS changes reported by Waldman [193] in two asymptomatic P102L patients; in one asymptomatic patient this could not be tested or detected.

For every patient diagnosed with inherited prion disease, there is a normal control population (1st degree relatives without the mutation) and a population who may be followed up regularly to check for symptom onset (mutation positive relatives or those not yet tested). My thesis suffers due to a lack of a control population, both for MRI examinations and clinical scores. Future studies should include a much larger, and potentially age and sex-matched (and environmentally matched in family members), control population. It is unlikely that many patients with prion disease are missed by the National Prion Clinic or the National CJD Surveillance Unit due to a National CJD referral agreement being in place as well as a heightened awareness of prion disease amongst neurologists and GPs because of its presence. Further steps may be taken to improve enrolment success by auditing reasons for patients turning down the trial and seeing if there are any solvable issues. Full training for staff,

including development of techniques to discuss the subject of the trial and recruitment into it should be mandatory.

The fact remains that prion disease is a rare, progressive and fatal disease in which patients and relatives may be unwilling to enter a clinical trial, particularly if they may be randomised to non-treatment arms of randomised therapeutic studies[209]. The emphasis for future trials must be to recruit as many patients as possible and to include normal controls as well as pre-symptomatic inherited patients and high risk pre-symptomatic vCJD exposed patients. Recruitment number aims ought to be realistic within the timeframe of the trial to answer all the questions. The National Prion Monitoring Cohort study addresses some of these issues, particularly in aiming to recruit more patients who might provide natural history control data. As no drug is being trialled, a further opportunity to describe the clinical and MRI features in prion disease will be provided. It is essential that prospective clinical and neuroimaging data are obtained, to establish criteria for early diagnosis, to understand the natural history of human prion disease, and to develop suitable outcome measures to evaluate new treatments as these become available.

5. CONCLUSIONS

5.1 ACHIEVEMENT OF AIMS

The range and pattern of cognitive and motor test performance in patients with prion disease and how these change with time have been described. Change in MTR at baseline and with time, has been quantified for the first time in a population with prion disease. Associations between clinical scores and MTR decline were demonstrated both at baseline and longitudinally.

5.2 HYPOTHESES

I have shown in this thesis that low MTR at baseline, as well as longitudinally, may be used as a biomarker of disease severity in prion disease. With the additional use of suggested severity scales in different types of prion disease, low cerebral MTR has potential as a prognostic indicator, and further studies necessary to fully establish the role of this marker are justified. Change in cerebral MTR has potential as an outcome measure in future clinical trials in prion disease.

APPENDICES

A PRION-1 MRI Scan Protocol

B Patient Information Sheet: PRION-1 MRI scans

C Standard Operating Procedure for MRI scans under General Anaesthesia in the PRION-1 trial

D Consent form for MRI examination

E PRION-1 trial MRI Proforma

F Proforma for DWI/FLAIR assessment

G MMSE (Mini Mental State Examination)

H CDR (Clinician's Dementia Rating Scale)

I ADAS-COG (Alzheimer's Disease Assessment Scale)

J GCS (Glasgow Coma Score)

K Barthel ADL

L CGIS (Clinician's Global Impression of disease Severity)

M Neurological Examination PRION-1 Form

N Cognitive Picture Tests

O Independent Neurologist Score sheet

P BPRS (Brief Psychiatric Rating Scale)

Q Longitudinal changes in MTR histogram measures and ROIs in P102L patients

R Longitudinal changes in MTR histogram measures and ROIs in 6 OPRI patients

S Summary of MT and clinical measures at baseline

T Tables showing slopes of change over time in MTR histogram and ROI parameters

U Tables showing slopes of change over time in MMSE, ADAS-COG, GCS, BPRS, and videoed cognitive and motor scores

V Associations between decline in MT measures and decline in clinical scores

Appendix A: PRION-1 MRI Scan Protocol

1. MRI Bookings

1.1 Trial Research Fellow to ensure that PRION-1 trial Investigations Consent form has been completed before each MRI is booked and carried out (Consent form does not need to be seen by MRI department).

1.2 Trial Nurse to book all MRI scans with Neuroradiology Department nurse at least 48hrs prior to scan being required.

1.3 Booking phone number and contact person : Trial Nurse XXXX

1.4 Request form and safety questionnaire to be completed before patient attends MRI appointment and given to MRI department by Trial Nurse at least 48 hours in advance.

1.5 MRI request forms and safety questionnaires to be available at National Prion Clinic.

1.6 Trial MRI scans to be carried out during the following times:

Inpatients:	Tuesday pm, Wednesday am
Outpatients:	Friday midday

1.7.1 At the time of making the booking the Radiographer should complete the PRION-1 Trial MRI Proforma with the aid of the Trial Nurse. A provisional decision as to how many sequences to attempt should be made by the Radiographer in the light of the proforma information.

1.8 If requested in advance, a Trial Research Fellow or Trial Nurse will accompany patient to MRI scan and stay with the patient until the scan is complete.

1.9 Inpatients will be transported from the ward by hospital porters who will also return them to the ward as per hospital protocol.
Outpatients will, depending on level of mobility and function, either attend alone, with a relative/carer or, if requested in advance, with the Trial Nurse. This will be confirmed at the time of booking.

1.10 The number of sequences actually performed will be at the discretion of the Radiologist or Radiographer depending on patient compliance and movement.

1.11 The NHS scanner at The National Hospital will be the only MRI scanner used.

1.12 The Radiographer will be responsible for checking the quality of the scan and re-scanning if necessary, as time permits.

1.13 The Neuroradiology Department must be notified immediately of any cancellations to MRI bookings.

2. Patient Preparation

2.1 MRI staff to ensure safety questionnaire has been filled in adequately and check there have been no changes since the form was signed. They should enquire about previous MRIs the patient may have had done, if none, then explain the procedure. Ask if the patient or their carer have any questions and answer them.

2.2 Establish whether oral sedation has been given, if required.

2.3 If any queries should arise contact the Trial Nurse for advice (see above).

3. MRI Scan Protocol

The MRI protocol will be programmed into the scanner and performed in the order of sequences below, as patient compliance permits:

Sequencing priority

1. Volumetric scan
2. Diffusion/ADC
3. DTI
4. FLAIR
5. MT
6. Spectroscopy

4. MRI Scan Data Collection, Analysis and Backup

4.1 Scans will be archived in the MRI Department as per standard protocol and also saved onto a designated 'PRION-1 Trial' optical disc to be provided, and replaced as necessary, by the Trial Research Fellow.

4.2 If requested by a Trial Research Fellow (and providing the network connection is open), scans will be 'pushed' onto the network by MRI staff.

4.3 After scanning the PRION-1 Trial MRI Proforma should be completed by the Radiographer showing which sequences were actually performed.

4.4 The Trial Research Fellow will take the completed forms to the National Prion Clinic for storage.

4.5 A hard copy of relevant sequences (eg FLAIR) will be produced and sent to Professor Yousry. Should any problems be identified the clinical team will be contacted. Prion-1 scans will not be reported routinely by the MRI Department.

Appendix B: Patient Information Sheet: PRION-1 MRI scans

Patient Information Sheets:
PRION-1: Clinical investigations

(Form to be on local headed paper)

Version: 2.0 Date: 12 January 2006

PRION-1: MRI

What is MRI?

MRI stands for Magnetic Resonance Imaging. This is a scanning procedure that uses a combination of a strong magnet, radiowaves and a computer to produce very detailed pictures of your body. The scan will not hurt and has no long-term effect on your body once it is over.

What does an MRI scan show?

An MRI scan provides pictures of the inside of your body. Whereas an ordinary x-ray produces very good pictures of the bones, an MRI scan can show details of the brain, muscles, nerves, cartilage and other internal organs.

Preparation for an MRI scan

As the MRI scanner uses a very strong magnet, there are some safety guidelines that must be followed. Let the staff in the scanning department know as soon as possible if any of the following applies to you:

- you have a pacemaker
- you have an artificial heart valve
- you have ever had surgery on your head or spine
- you have any metallic implants, for example joint replacement
- you have ever had metal in your eyes, for example from welding or metalwork
- you may be pregnant

In some of these cases you may need to have an X-ray to make sure that it is safe for you to have an MRI scan. The staff in the MRI Department will discuss this with you. You will also be asked to remove personal belongings such as your watch, jewellery, keys, credit cards and coins. This is because if you go into the scan room with loose metal objects in your pockets they may be pulled out by the strong magnetic field and fly into the scanner. If you wear your watch into the scanner it may not work when you come out and if you have credit cards in your pocket the information held on the magnetic strip will be wiped off. Metal fastenings on your clothes are all right because the magnetic field is not strong enough to pull them off. However, if they are close to the part of your body you are having scanned they may interfere with the pictures and you may be asked to change into a gown.

Involuntary movements such as muscle jerking, which is common in prion disease, can interfere with the MRI scan. If this is likely to be a problem with your scan, the study doctor will discuss with you about having sedation before the scan. This sedation may either be taken in tablet form or as a general anaesthetic. If the MRI scan were to be performed under a general anaesthetic it carries the usual risks associated with receiving general anaesthetic.

What happens during the scan?

You will be asked to lie on the scanner couch where you will be made as comfortable as possible. The position will vary depending on the part of the body that is being scanned. For example, for a scan of the head

Appendix C: Standard Operating Procedure for MRI scans under General Anaesthesia in the PRION-1 trial

STANDARD OPERATING PROCEDURE FOR MRI SCANS DONE UNDER GENERAL ANAESTHESIA (GA) AT THE NATIONAL HOSPITAL FOR NEUROLOGY AND NEUROSURGERY (NHNN) FOR THE NATIONAL PRION CLINIC (NPC)

1. Patients with different forms of prion disease will be scanned under general anaesthetic, where patient movement would otherwise prevent scans of adequate quality being obtained.

2. The procedure will be discussed with patients, or their next of kin (in cases where patients are cognitively impaired and lack capacity to consent), as far as possible in advance by one of the trial fellows or the trial nurse. Information about the procedure will be provided and intended benefits and risks explained. In addition, in cognitively impaired patients, written assent will be obtained from next of kin in advance of the scan appointment. This procedure will be repeated at follow-up trial visits. The next of kin may be referred to Dr. Sally Wilson, consultant anaesthetist, NHNN (email: if they request a detailed discussion directly with the anaesthetist.

3. Dr. Sally Wilson at NHNN or air call through NHNN switchboard) and Caroline Andrews, superintendent radiographer, MRI Department, NHNN at NHNN) will be informed (where possible) two weeks in advance by one of the trial nurses (Christopher Rhymes, email:

4. MRI scans will be done under GA on Wednesday afternoons at 2:30 pm. Patients will usually be admitted for planned assessments on Tuesday to the Nuffield Ward at NHNN and stay for 2 nights. Dr. Sally Wilson will consent those patients on the ward who are able to give consent themselves. She will be assisted by one of the trial or clinical research fellows (Dr. D. Siddique, email:

5. Patients will be kept overnight after MRI scan under GA has been performed. Out of hours neurology cover will be provided by the on-call neurology SHO and SpR, and anaesthetic cover will be provided by the on-call anaesthetic SpR (bleep 8131). Dr Sally Wilson will be available out of hours through switchboard in case of an emergency.

6. All patients having MRI scan under GA will be resuscitated in the event of a cardiorespiratory arrest during or after the procedure.

Appendix D: Consent form for MRI examination

Fill in with Forms 2, 3 and 5	PRION-1 TRIAL TRIAL INVESTIGATIONS CONSENT	MRC PRION-1

Investigations Consent Date: Day ☐☐ Month ☐☐ Year 2 0 0 ☐ Male ○ Female ○ Initials ☐☐☐ Soundex ☐☐☐☐

Date of Birth: ☐☐ ☐☐ 1 9 ☐☐ Hospital Number ☐☐☐☐☐☐☐

PRION-1 Study Number ☐☐☐☐☐☐

Randomised to immediate quinacrine ○ Quinacrine ○
Randomised to deferred quinacrine ○ No quinacrine ○

Visit month number 0□ 1□ 2□ 4□ 6□ 9□ 12□ 15□ 18□ 21□ 24□ Extra visit □

PRION-1 TRIAL INVESTIGATIONS CONSENT

Please initial box if you agree to the following:

1. I agree to having an MRI brain scan ☐

2. I agree to having an electroencephalogram (EEG) ☐

3(a) I agree to having a lumbar puncture and CSF sample taken for testing. ☐

3(b) I agree to CSF samples being stored and assign all right, title and interest in such samples to the Medical Research Council. I confirm that it has been explained to me that the stored samples may also be used for research or teaching purposes and that they may be used to develop commercial diagnostic or therapeutic agents. ☐

4. I agree to having a tonsil biopsy ☐

Individual's signature	Print name	Date

Carer's signature (where relevant)	Print name	Date

I have explained the nature, demands and foreseeable risks of these investigations to the patient

Investigator's signature	Print name	Date

IMPORTANT: **One signed original to be given to patient or their representative**
One signed original to be kept on file by the researcher
One signed original to be kept in the clinic notes

CRF Version 2.0, January 2006

Appendix E: PRION-1 trial MRI Proforma

To be completed for each scan for every trial patient with aid of Prion-1 Trial Nurse (contact on 020 7405 0755 (Prion Office) or pager 08700555500 #H4757 (Prion Nurse specialist) if any queries arise)

Name:

Date of scan: / /

Please tick as appropriate:

Sequencing priority	Anticipated sequences possible	Actual sequences performed
Volumetric scan (10 mins)		
Diffusion/ADC (1 min)		
DTI (10 mins)		
FLAIR (4 mins)		
MT (12 mins)		
Spectroscopy		

Hard copy generated? Yes / No

Archived onto Prion-1 Trial Optical Disc? Yes / No

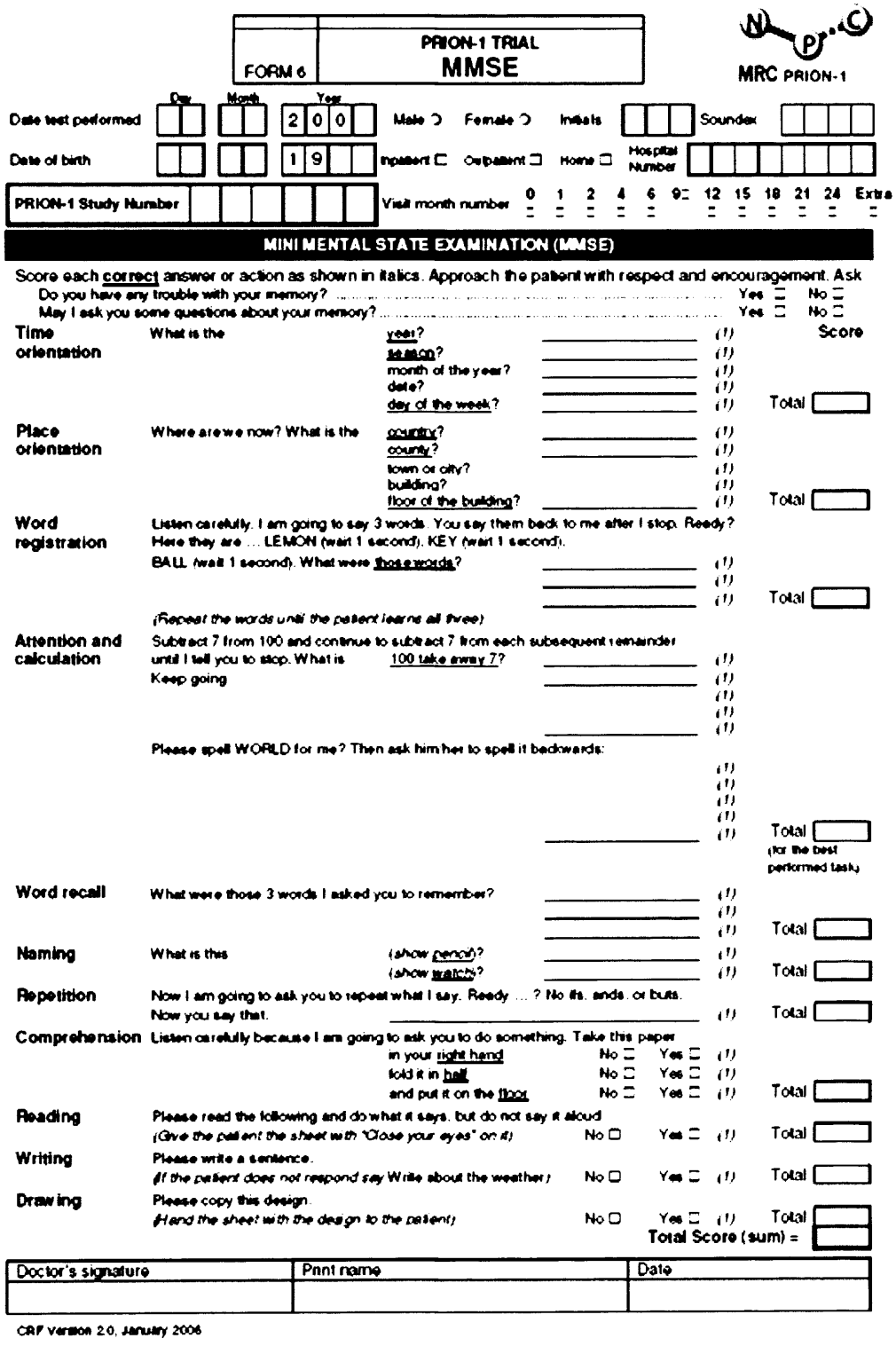
Radiographer signature..... Date.....

Appendix F: Proforma for DWI/FLAIR assessment

NAME	DATE	CODE	DIFFUSION-WEIGHTED IMAGING													
			NORMAL	ABNORMAL												
				CAUDATE	PUTAMEN	GLOBUS PALLIDUS	THALAMUS	FRONTAL CORTEX	PARIETAL CORTEX	TEMPORAL CORTEX	OCCIPITAL CORTEX	INSULAR CORTEX	CINGULATE CORTEX			

NAME	DATE	CODE	FLAIR IMAGING													
			NORMAL	ABNORMAL												
				CAUDATE	PUTAMEN	GLOBUS PALLIDUS	THALAMUS	FRONTAL CORTEX	PARIETAL CORTEX	TEMPORAL CORTEX	OCCIPITAL CORTEX	INSULAR CORTEX	CINGULATE CORTEX			

Appendix G: MMSE (Mini Mental State Examination)



FORM 6 — PRION-1 TRIAL — MMSE — MRC PRION-1

Date test performed (Day / Month / Year 2 0 0) Male ○ Female ○ Initials Soundex

Date of birth (1 9) Inpatient ☐ Outpatient ☐ Home ☐ Hospital Number

PRION-1 Study Number — Visit month number 0 1 2 4 6 9☐ 12 15 18 21 24 Extra

MINI MENTAL STATE EXAMINATION (MMSE)

Score each correct answer or action as shown in italics. Approach the patient with respect and encouragement. Ask

Do you have any trouble with your memory? ... Yes ☐ No ☐

May I ask you some questions about your memory? ... Yes ☐ No ☐

					Score
Time orientation	What is the	year?	_____	(1)	
		season?	_____	(1)	
		month of the year?	_____	(1)	
		date?	_____	(1)	
		day of the week?	_____	(1)	Total ☐
Place orientation	Where are we now? What is the	country?	_____	(1)	
		county?	_____	(1)	
		town or city?		(1)	
		building?		(1)	
		floor of the building?	_____	(1)	Total ☐
Word registration	Listen carefully. I am going to say 3 words. You say them back to me after I stop. Ready? Here they are ... LEMON (wait 1 second), KEY (wait 1 second), BALL (wait 1 second). What were those words?		_____	(1)	
			_____	(1)	
			_____	(1)	Total ☐
	(Repeat the words until the patient learns all three)				
Attention and calculation	Subtract 7 from 100 and continue to subtract 7 from each subsequent remainder until I tell you to stop. What is	100 take away 7?	_____	(1)	
	Keep going		_____	(1)	
				(1)	
				(1)	
			_____	(1)	
	Please spell WORLD for me? Then ask him/her to spell it backwards:			(1)	
				(1)	
				(1)	
				(1)	
			_____	(1)	Total ☐ (for the best performed task)
Word recall	What were those 3 words I asked you to remember?		_____	(1)	
			_____	(1)	
			_____	(1)	Total ☐
Naming	What is this	*(show pencil)*?	_____	(1)	
		(show watch)?	_____	(1)	Total ☐
Repetition	Now I am going to ask you to repeat what I say. Ready ... ? No ifs, ands, or buts. Now you say that.		_____	(1)	Total ☐
Comprehension	Listen carefully because I am going to ask you to do something. Take this paper				
		in your right hand	No ☐ Yes ☐	(1)	
		fold it in half	No ☐ Yes ☐	(1)	
		and put it on the floor	No ☐ Yes ☐	(1)	Total ☐
Reading	Please read the following and do what it says, but do not say it aloud *(Give the patient the sheet with "Close your eyes" on it)*		No ☐	Yes ☐ (1)	Total ☐
Writing	Please write a sentence. *(If the patient does not respond say* Write about the weather*)*		No ☐	Yes ☐ (1)	Total ☐
Drawing	Please copy this design. *(Hand the sheet with the design to the patient)*		No ☐	Yes ☐ (1)	Total ☐
					Total Score (sum) = ☐

Doctor's signature	Print name	Date

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Appendix H: CDR (Clinician's Dementia Rating Scale)

FORM 11 | PRION-1 TRIAL CDR

MRC PRION-1

Date test performed: Day | Month | Year 2 0 0 | Male ○ Female ○ | Initials | Soundex

Date of birth: 1 9 | Inpatient □ Outpatient □ Home □ | Hospital Number

PRION-1 Study Number

Visit month number: 0 1 2 4 6 9 12 15 18 21 24 Extra

CLINICIAN'S DEMENTIA RATING (CDR)

Each of the 6 domains should be rated by circling the number by the term that best describes the patient's current capabilities. Rate each domain as independently as possible, as it is not unusual for patients to have varying degrees of impairment across domains. Only assign scores greater than 0 if impairment is due to cognitive loss.

			Score
Memory	No memory loss or slight inconstant forgetfulness	0	
	Mild consistent forgetfulness; partial recollection of events; "benign" forgetfulness	0.5	
	Moderate memory loss; more marked for recent events; defect interferes with everyday activities	1	
	Severe memory loss; only highly learned material retained; newly material rapidly lost	2	
	Severe memory loss; only fragments remain	3	
Orientation	Fully orientated	0	
	Fully orientated except for slight difficulty with time relationships	0.5	
	Moderate difficulty with time relationships; orientated for place at examination, but may have geography disassociation elsewhere	1	
	Severe difficulty with time relationships; usually disorientated in time, often in place	2	
	Orientated to person only	3	
Judgement and problem solving	Solves everyday problems well; judgement good in relation to past performance	0	
	Only slight impairment in solving problems, similarities, differences	0.5	
	Moderate difficulty in handling problems, similarities, differences; social judgement usually maintained	1	
	Severely impaired in handling problems, similarities, differences; social judgement usually impaired	2	
	Unable to make judgements or solve problems	3	
Community affairs	Independently functions at usual level in job, shopping, business and financial affairs, volunteer and social groups	0	
	Slight impairment in these activities	0.5	
	Unable to function independently at these activities although may still be engaged in some; appears normal to casual inspection	1	
	No pretence of independent function outside home; appears well enough to be taken to functions outside family home	2	
	No pretence of independent function outside home; appears too ill to be taken outside family home	3	
Home and hobbies	Life at home, hobbies, intellectual interests well maintained	0	
	Life at home, hobbies, intellectual interests well slightly impaired	0.5	
	Mild but definite impairment of function at home; more difficult chores abandoned; more complicated hobbies and interests abandoned	1	
	Only simple chores preserved; very restricted interests, poorly sustained	2	
	No significant function at home	3	
Personal care	Fully capable of self care	0	
	Needs prompting	1	
	Requires assistance in dressing, hygiene, keeping of personal effects	2	
	Requires substantial help with personal care; frequently incontinent	3	
		Total Score (sum) =	

Doctor's signature	Print name	Date

CRF Version 2.0, January 2006

Appendix I: ADAS-COG (Alzheimer's Disease Assessment Scale)

FORM 10 | PRION-1 TRIAL
ADAS-COG

MRC PRION-1

Date test performed: Day / Month / Year 2 0 0 | Male ○ Female ○ | Initials | Sounder

Date of birth: 1 9 | Inpatient ☐ Outpatient ☐ Home ☐ | Hospital Number

PRION-1 Study Number | Visit month number: 0 1 2 4 6 9 12 15 18 21 24 Extra

ALZHEIMER'S DISEASE ASSESSMENT SCALE (ADAS-COG)

Score each incorrect answer or action in each of the 11 domains below.

1. Word recall

I am going to show you some words, one at a time. Please read each word out loud and try to remember it, because later I will ask you to try to remember all of the words I have shown you. *(The patient reads aloud 10 words exposed for 2 seconds each. The patient then recalls the words aloud. Three trials of reading and recalling are given.)*

Trial 1	Recalled	Not recalled	Trial 2	Recalled	Not recalled	Trial 3	Recalled	Not recalled
Butter	☐	☐	Pole	☐	☐	Shore	☐	☐
Arm	☐	☐	Letter	☐	☐	Letter	☐	☐
Shore	☐	☐	Butter	☐	☐	Arm	☐	☐
Letter	☐	☐	Queen	☐	☐	Cabin	☐	☐
Queen	☐	☐	Arm	☐	☐	Pole	☐	☐
Cabin	☐	☐	Shore	☐	☐	Ticket	☐	☐
Pole	☐	☐	Grass	☐	☐	Engine	☐	☐
Ticket	☐	☐	Cabin	☐	☐	Grass	☐	☐
Grass	☐	☐	Ticket	☐	☐	Butter	☐	☐
Engine	☐	☐	Engine	☐	☐	Queen	☐	☐
Total not recalled			Total not recalled			Total not recalled		

Score = mean number of words not recalled on 3 trials (maximum = 10) =

2. Naming objects and fingers

I am going to show you some objects. What is this called? or What is the name of this thing? *(If the patient does not respond, give the cue listed below. If the patient still does not respond or makes an error go onto the next object).*

Objects	Standard clue that can be used to assist those patients having difficulties	Correct	Incorrect (or not named)
Flower	- grows in the garden	☐	☐
Bed	- used for sleeping	☐	☐
Whistle	- makes a sound when you blow it	☐	☐
Pencil	- used for writing	☐	☐
Rattle	- a baby's toy	☐	☐
Mask	- hides your face	☐	☐
Scissors	- cuts paper	☐	☐
Comb	- used on hair	☐	☐
Wallet	- holds your money	☐	☐
Harmonica	- a musical instrument	☐	☐
Stethoscope	- doctor uses it to listen to your heart	☐	☐
Tweezers	- used to pick up things	☐	☐
Now can you name the fingers on your left/right *(dominant)* hand?		☐	☐
Thumb		☐	☐
Index		☐	☐
Middle		☐	☐
Ring		☐	☐
Little		☐	☐
		Total incorrect	

Score (0=0-2 incorrect; 1=3-5 incorrect; 2=6-8 incorrect; 3=9-11 incorrect; 4=12-14 incorrect; 5=15-17 incorrect) =

CRF Version 2.0, January 2006

Rosen W.G., Mohs R.C., Davis K.L. Am J Psychiatry 1984; 141:11:1356-1364.

3. Commands

I am going to ask you to do some actions. *(Each command should be read once. If the patient does not respond, or makes an error, give the command one more time. Then go onto the next command. All commands should be given.)*

Command *(Each underlined element represents a single step. Each command is scored as a whole)*	Correct	Incorrect (or not performed)
Make a fist	☐	☐
Point to the ceiling and then to the floor	☐	☐
Line up a pencil, watch, and card, in that order, on a table in front of the patient		
Put the pencil on top of the card and then put it back	☐	☐
Put the watch on the other side of the pencil and then turn over the card	☐	☐
Tap each shoulder twice, with two fingers, keeping your eyes shut	☐	☐

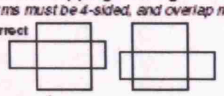
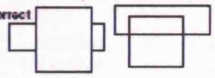
Score = number of incorrectly performed commands (minimum 0, maximum 5) = ☐

4. Constructional praxis

On this paper is a shape. Try to draw another one that looks just like this, somewhere on the page.
(Allow 2 attempts for each shape, then go onto the next shape. A drawing should be scored as correct if the patient has reproduced all the essential geometric features of the original. Changes in size do not count as errors. Small gaps between lines do not indicate an error, as long as the shape has been reproduced.)

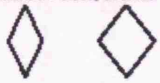
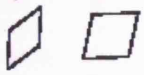
Shape	Correct	Incorrect (or not drawn)
Circle *(a closed curved figure)*	☐	☐
Two overlapping rectangles	☐	☐

(forms must be 4-sided, and overlap must be similar to presented form. Changes in size are not scored.)

Correct  Incorrect 

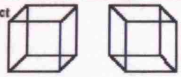
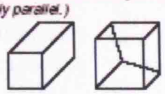
Diamond	☐	☐

(Figure must be 4-sided, oriented with points at the top and bottom, and the sides are approximately equal length.)

Correct  Incorrect 

Cube	☐	☐

(The form is 3-dimensional, with front face in the correct orientation, internal lines drawn correctly between corners. Opposite sides of faces should be approximately parallel.)

Correct  Incorrect 

Total ☐ **correct**

Score = number of incorrectly drawn figures (score=5 if only scribbles, parts of shapes, or words) = ☐

5. Ideational praxis

I want you to pretend that you have written yourself a letter. Take this piece of paper, fold it so that it will fit into the envelope, and then put it into the envelope. Then seal the envelope, address the envelope to yourself, and show me where the stamp goes. *(If the patient forgets part of the task, or is having difficulty, repeat the instruction for the component of the task where the patient is having difficulty.)*

Command	Correct	Incorrect (or not performed)
Fold a letter	☐	☐
Put the letter in an envelope	☐	☐
Seal the envelope	☐	☐
Address the envelope	☐	☐
Indicate where the stamp goes	☐	☐

Score = number of incorrectly performed commands = ☐

6. Orientation

Ensure that no clocks, watches, or calendars are visible.

	Correct	Incorrect (or not answered)		Correct	Incorrect (or not answered)
Full name *(first and last)*	☐	☐	Year	☐	☐
Day	☐	☐	Season *(within 1 week of upcoming or 2 weeks of previous season)*	☐	☐
Date *(± 1 day)*	☐	☐	Time of day *(± 1 hour)*	☐	☐
Month	☐	☐	Place *(partial or full name of site)*	☐	☐

Score = number of incorrect answers = ☐

7. Word recognition

FORM 10	PRION-1 TRIAL **ADAS-COG**

MRC PRION-1

I am going to show you some words printed on cards. I want you to read each word out loud and try to remember it. *(If the patient cannot read a word, say the word out loud. However, it is important for the patient to actually look at each word and try to read it.)* Now I'm going to show you another set of words. Some of the words were on the list I just showed you, and others are new. For each word, I want you to tell me whether it is one of the words I just showed you. Is this one of the words I showed you before, yes or no? *(or Did I show you this word before?) (The same instruction is given before the second test word. For the remaining test words say How about this one?) (If the patient does not remember the task (eg reads the word rather than responding "Yes" or "No"), then repeat or rephrase the entire question and make a note that the patient had to be reminded.)*

Trial 1	Yes/ Shown	No/ New	Remind	Trial 2	Yes/ Shown	No/ New	Remind	Trial 3	Yes/ Shown	No/ New	Remind
Nurse	○	□	□	**Board**	□	○	□	**Coin**	□	○	□
Magazine	□	○	□	*Turnip*	○	□	□	*Plank*	○	□	□
Wizard	□	○	□	**Gem**	□	○	□	*War*	○	□	□
Van	□	○	□	**Institution**	□	○	□	*Porch*	○	□	□
Leopard	□	○	□	**Coin**	□	○	□	*Toast*	○	□	□
Safe	○	□	□	*Master*	○	□	□	*Rope*	○	□	□
Sea	□	○	□	**Magazine**	□	○	□	**Anchor**	□	○	□
Train	□	○	□	**Van**	□	○	□	**Board**	□	○	□
Coin	□	○	□	**Anchor**	□	○	□	**Leopard**	□	○	□
Ship	○	□	□	*Lumber*	○	□	□	*Judge*	○	□	□
Institution	□	○	□	*Servant*	○	□	□	**Magazine**	□	○	□
Map	○	□	□	*Pond*	○	□	□	*Camp*	○	□	□
Axe	○	□	□	*Military*	○	□	□	**Sea**	□	○	□
Board	□	○	□	*Hospital*	○	□	□	**Institution**	□	○	□
Carrot	○	□	□	**Sea**	□	○	□	*Tack*	○	□	□
Milk	○	□	□	*Jungle*	○	□	□	*Emerald*	○	□	□
Volume	○	□	□	*Nail*	○	□	□	**Van**	□	○	□
Forest	○	□	□	**Wizard**	□	○	□	*Globe*	○	□	□
Anchor	□	○	□	**Leopard**	□	○	□	**Train**	□	○	□
Gem	□	○	□	**Train**	□	○	□	**Fund**	□	○	□
Cat	○	□	□	*Editorial*	○	□	□	*Coast*	○	□	□
Fund	□	○	□	*Bread*	○	□	□	**Gem**	□	○	□
Edge	○	□	□	**Fund**	□	○	□	**Wizard**	□	○	□
Cake	○	□	□	*Trade*	○	□	□	*Kitten*	○	□	□
Total circles ticked (incorrect response)				**Total circles ticked** (incorrect response)				**Total circles ticked** (incorrect response)			

Score = mean number of incorrect words on 3 trials (max=12; score 12 if average more than 12 incorrect responses) =

8. Remembering test instructions

Evaluate the patient's ability to remember the requirements of the word recognition task above.

0 did not need any extra reminders of instructions
1 very mild – forgot once
2 mild – reminded twice
3 moderate – reminded 3 or 4 times
4 moderately severe – reminded 5 or 6 times
5 severe – reminded 7 or more times

Score =

9. Spoken language ability

Provide a global rating of the quality of speech, ie clarity, difficulty in making oneself understood.

0 no instance where it is difficult to understand the patient
1 very mild – one instance of lack of understandability
2 mild – patient has difficulty less than 25% of time
3 moderate – patient has difficulty 25-50% of time
4 moderately severe – patient has difficulty more than 50% of time
5 severe – one or two word utterance; fluent, but empty speech; mute

Score =

Rosen W.G., Mohs R.C., Davis K.L. Am J Psychiatry 1984; 141:11:p1356-1364

10.Word-finding difficulty in spontaneous speech
Rate the patient's difficulty in finding desired words, e.g., circumlocutions.

- **0** no evidence of word-finding difficulty in spontaneous speech
- **1** very mild – 1 or 2 instances, not clinically significant
- **2** mild – noticeable circumlocution or synonym substitution
- **3** moderate – loss of words without compensation on occasion
- **4** moderately severe – frequent loss of words without compensation
- **5** severe – nearly total loss of content words; speech sounds empty; 1-2 words utterances

Score = ☐

11.Comprehension
Rate the patient's ability to understand speech. Do not include responses to commands.

- **0** no evidence of poor comprehension
- **1** very mild – 1-2 instances of misunderstanding
- **2** mild – 3-5 instances of misunderstanding
- **3** moderate – requires several repetitions and rephrasing
- **4** moderately severe – patient only occasionally responds correctly; e.g., yes/no questions
- **5** severe – patient rarely responds to questions appropriately, not due to poverty of speech

Score = ☐

12.Concentration/distractibility
Rate the frequency with which the patient is distracted by irrelevant stimuli and/or must be reoriented to the ongoing task because the patient has lost his/her train of thought or appears to be caught up in his/her own thoughts.

- **0** no evidence of poor concentration or distractibility
- **1** very mild – one instance of poor concentration
- **2** mild – 2-3 instances of poor concentration/distractibility; signs of restlessness and inattentiveness
- **3** moderate – 4-5 instances during interview
- **4** moderately severe – poor concentration/distractibility throughout much of interview
- **5** severe – extreme difficulty in concentration and extremely distractible, unable to complete tasks

Score = ☐

SCORE SUMMARY

Copy the scores in each domain from the grey boxes, then total.

1 Word recall *(max 10)* ☐	5 Ideational praxis *(max 5)* ☐	9 Spoken language ability *(max 5)* ☐
2 Naming objects and fingers *(max 5)* ☐	6 Orientation *(max 8)* ☐	10 Word-finding difficulty in spontaneous speech *(max 5)* ☐
3 Commands *(max 5)* ☐	7 Word recognition *(max 12)* ☐	11 Comprehension *(max 5)* ☐
4 Constructional praxis *(max 5)* ☐	8 Remembering test instructions *(max 5)* ☐	12 Concentration/ distractability *(max 5)* ☐

Total score *(max 75)* ☐

Doctor's signature	Print name	Date

CRF Version 2.0, January 2006

Appendix J: GCS (Glasgow Coma Score)

FORM 13

PRION-1 TRIAL

GLASGOW COMA SCORE

MRC PRION-1

Date test performed: Day / Month / Year 2 0 0 | Male ○ Female ○ | Initials | Soundex

Date of birth: 1 9 | Inpatient ☐ Outpatient ☐ Home ☐ | Hospital Number

PRION-1 Study Number

Visit month number: 0 1 2 4 6 9 12 15 18 21 24 Extra

GLASGOW COMA SCORE

Each of the 3 responses should be rated on scales.
Circle the number headed by the term that best describes the patient's current response.

			Score
Eyes open	Spontaneously	4	
	To speech	3	
	To pain	2	
	None	1	
Verbal response	Orientated	5	
	Confused (sentences)	4	
	Inappropriate words	3	
	Incomprehensible sounds	2	
	None	1	
Best motor response (record best arm)	Obeys commands	6	
	Localise pain	5	
	Normal flexion (withdraws from pain)	4	
	Abnormal flexion	3	
	Extension to pain	2	
	None	1	
		Total Score (sum) =	

Doctor's signature	Print name	Date

CRF version 2.0, January 2006

Appendix K: Barthel ADL

FORM 9 | PRION-1 TRIAL
BARTHEL ADL

MRC PRION-1

Date test performed: Day ☐☐ Month ☐☐ Year 2 0 0 ☐ | Male ○ Female ○ | Initials ☐☐☐ | Soundex ☐☐☐☐

Date of birth: ☐☐ ☐☐ 1 9 ☐☐ | Inpatient ☐ Outpatient ☐ Home ☐ | Hospital Number ☐☐☐☐☐☐☐

PRION-1 Study Number ☐☐☐☐☐☐ | Visit month number: 0 1 2 4 6 9 12 15 18 21 24 Extra

MODIFIED BARTHEL ACTIVITIES OF DAILY LIVING (ADL)

A) Bowels Score = ☐
- 0: Incontinent or needs enema
- 1: Occasional accident
- 2: Continent

B) Bladder Score = ☐
- 0: Incontinent or catheterised
- 1: Occasional accident
- 2: Continent for > 7 days

C) Grooming Score = ☐
- 0: Needs help with personal care
- 1: Independent with aids if necessary

D) Toilet use Score = ☐
- 0: Dependent
- 1: Needs some help
- 2: Fully independent

E) Feeding Score = ☐
- 0: Unable
- 1: Needs help
- 2: Independent

F) Transfers Score = ☐
- 0: Unable, no sitting balance
- 1: Major help (one or two people, physical), can sit
- 2: Minor help (verbal or physical)
- 3: Independent

G) Mobility Score = ☐
- 0: Immobile
- 1: Wheelchair – independent
- 2: Walk with help of one person
- 3: Independent

H) Dressing Score = ☐
- 0: Dependent
- 1: Needs help
- 2: Independent

I) Stairs Score = ☐
- 0: Unable
- 1: Needs help
- 2: Independent

J) Bathing Score = ☐
- 0: Dependent
- 1: Independent

Total Score (sum, max 20) = ☐

CAREGIVER INTERVIEW SUMMARY

1. Name of caregiver completing questions _____

2. Relationship to patient _____

3. *[Do not complete at trial/study entry]*
Circle the **one** descriptor that best characterises the caregiver's global impression of the patient's **current** condition relative to the patient's condition at **baseline** (trial/study entry).

1 Markedly improved
2 Moderately improved
3 Minimally improved
4 Unchanged
5 Minimally worse
6 Moderately worse
7 Markedly worse

Note: **minimal** means there should be a "**detectable**" change in the patient;
moderate that the degree of change should be "**clearly apparent**";
marked that the degree of change should be considered "**dramatic**".

4. With respect to the patient's symptoms, which are the four biggest problems for you and/or other caregivers? Please include **all** problems, not just those related to bodily functions, but also mood, attitude, general behaviour. (More than one symptom can be listed, but please rank with most problematic first, indicate how much each troubles you in the tick boxes, and give the best descriptor of how this problem has changed since trial/study entry using the scores in Question 3)

	not at all troubling	mildly troubling	moderately troubling	severely troubling	Score
1 _____	☐	☐	☐	☐	☐
2 _____	☐	☐	☐	☐	☐
3 _____	☐	☐	☐	☐	☐
4 _____	☐	☐	☐	☐	☐

CRF Version 2.0, January 2006

Appendix L: CGIS (Clinician's Global Impression of disease Severity)

FORM 12 | PRION-1 TRIAL
GLOBAL IMPRESSION OF CHANGE
MRC PRION-1

Date test performed: Day ☐☐ Month ☐☐ Year 2 0 0 ☐ | Male ○ Female ○ | Initials ☐☐☐ | Soundex ☐☐☐☐

Date of birth: ☐☐ ☐☐ 1 9 ☐☐ | Inpatient ☐ Outpatient ☐ Home ☐ | Hospital Number ☐☐☐☐☐☐☐

PRION-1 Study Number ☐☐☐☐☐☐ | Visit month number: 0 1 2 4 6 9 12 15 18 21 24 Extra

RATING OF GLOBAL SEVERITY

Each rater should select the **one** descriptor which best characterises your global impression of the patient's **current** condition. Your rating should be carried out independently, but be based on information derived from review of patient records and other study assessments and may note information elicited from other study personnel (CIBIC-plus).

1 Normal, not ill at all
2 Borderline mentally ill
3 Mildly ill
4 Moderately ill
5 Markedly ill
6 Severely ill
7 Amongst the most ill

		Day	Month	Year		
Doctor	Date of Rating	☐☐	☐☐	2 0 0 ☐	Rating ☐	Rater's Initials ☐☐☐
Nurse	Date of Rating	☐☐	☐☐	2 0 0 ☐	Rating ☐	Rater's Initials ☐☐☐

RATING of CHANGE from BASELINE

Select the **one** descriptor which best characterises your global impression of the patient's **current** condition relative to the patient's condition at **baseline** (trial/study entry). Your rating should be carried out independently, and be based **only** on information derived from your **interviews with both the patient and the caregiver**, with reference to your notes from the baseline interview (CIBIC-plus).

1 Markedly improved
2 Moderately improved
3 Minimally improved
4 Unchanged
5 Minimally worse
6 Moderately worse
7 Markedly worse

Note: mild means there should be a "detectable" change in the patient; moderate that the degree of change should be "clearly apparent"; and marked that the degree of change should be considered "dramatic".

		Day	Month	Year		
Doctor	Date of Rating	☐☐	☐☐	2 0 0 ☐	Rating ☐	Rater's Initials ☐☐☐
Nurse	Date of Rating	☐☐	☐☐	2 0 0 ☐	Rating ☐	Rater's Initials ☐☐☐

RATING of CHANGE from LAST ASSESSMENT

This following section should be completed by the doctor completing the sections above
Using the same scale, select the **one** descriptor which best characterises the composite global impression of all the clinical and nursing staff of the patient's **current** condition relative to the patient's condition at **the last assessment**.

		Day	Month	Year		
Overall	Date of Rating	☐☐	☐☐	2 0 0 ☐	Rating ☐	Rater's Initials ☐☐☐
Cognitive Performance	Date of Rating	☐☐	☐☐	2 0 0 ☐	Rating ☐	Rater's Initials ☐☐☐
Motor skills	Date of Rating	☐☐	☐☐	2 0 0 ☐	Rating ☐	Rater's Initials ☐☐☐
Psychiatric Status	Date of Rating	☐☐	☐☐	2 0 0 ☐	Rating ☐	Rater's Initials ☐☐☐

Doctor's signature	Print name	Date

Nurse's signature	Print name	Date

CRF Version 2.0, January 2006

FORM 7	PRION-1 TRIAL NEUROLOGICAL EXAMINATION

MRC PRION-1

Date form completed: Day ☐☐ Month ☐☐ Year 2 0 0 ☐ Male ○ Female ○ Initials ☐☐☐ Soundex ☐☐☐☐

Date of birth: ☐☐ ☐☐ 1 9 ☐☐ Hospital Number ☐☐☐☐☐☐☐☐

PRION-1 Study Number ☐☐☐☐☐☐ Video ID number ☐☐☐☐ Visit month number 0 = 1 = 2 = 4 = 6 = 9 = 12 = 15 = 18 = 21 = 24 = Extra =

NEUROLOGICAL EXAMINATION

1. **Is the neurological examination being digitally recorded** Yes ☐ No ☐ **If No, reason** ..... **Full video** ☐ **Severely affected protocol** * ☐
2. **Cognitive Function** (For all tests: Tick if correct, cross if incorrect, leave blank if not tested, U if unable to quantify)

No.	Task	Standard Question	Workings	Number Correct
1.*	Memory	"What is your name? What is your age? Which month is your birthday in?"	name / age / month	/ 3
2.	Letter cancelling	"Can you show me all the letter A / B / E's?"		/ 12
3.	Line drawings	"Can you tell me what these drawings are?"	scorpion/ tricycle/ spade/ owl/ violin/ hippo/ ladybird/ peg/ squirrel/ padlock beetle/ trolley/ spanner/ cockerel/ guitar/ camel/ butterfly/ nail/ goat/ door handle crab/ skateboard/ basket/ duck/ harp/ kangaroo/ spider/ saucepan/ pins/ sheep/ key	/ 10
4.	Reading passage	"Can you read this passage out for me? I will ask you some questions about it later."	dysphasia / poor fluency / neologism	norm/mild/mod/sev
5.	Spelling	"Can you spell the word...as in..."	aunt / eye / build / humour / neighbour / autumn	/ 6
6.	Fragmented letters	"Can you see any letters here?"	E / N / K L / M / P A / C / W	/ 3
7.	Fragmented objects	"Can you see any objects here?"	teapot / shoe / chair / guitar or violin pig / cake / boot / car fish / boat / plane / dog	/ 4
8.	Calculation	"I'd like you to do some sums for me. What is..."	5+4(9), 7+5(12), 13+8(21), 17+25(42)	/ 4
9.	Miming	"Show me how you."	brush teeth / comb hair / use a screwdriver	/ 3
10.	Copying gestures	"Can you copy these shapes for me with your hand?"	'ring' / 'horns' / 'three prongs'	/ 3
11.	Frontal lobe sequencing	"Can you copy this for me?"	-	norm/mild/mod/sev
12.	Words beginning with the letter	"Can you give me as many words beginning with the letter F / A / S as possible?" (Choose one only)		
13.	Proverbs	"Can you tell me what these sayings mean?"	strike while the iron / too many cooks	/ 2
14.	Digit span	"Can you repeat these numbers after me?"	5-9-2, 6-4-3-9, 4-2-7-3-1, 6-1-9-4-7-3, 5-9-1-7-4-2-8	/ 5
15.	Recall	"Tell me everything you can remember about the passage you read out to me"		norm/mild/mod/sev

Motor Function

No.	Task	Standard question	Right	Left	Example
16.*	Eye movements	"Keep your head still and follow my finger with your eyes"	norm / nystagmus / failure of upgaze / other		
17.	Finger-nose testing	Put your index finger on your nose....then on my finger...and keep going backwards and forwards between the two"...and now with the other hand"	norm / mild / mod / sev	norm / mild / mod / sev	
18.	Rapid alternating hand movements	"Can you copy this for me?...and with the other hand"	norm / mild / mod / sev	norm / mild / mod / sev	
19.	Sequential index finger tapping	"Can you do this for me?" "And with the other hand"	norm / mild / mod / sev	norm / mild / mod / sev	
20.	Sequential opposition	"Can you do this for me?" "And with the other hand"	norm / mild / mod / sev	norm / mild / mod / sev	
21.*	Primitive reflexes	"Could you look straight ahead for me? I am just going to tap on your forehead...and now on your lip...and look at the camera while I stroke your hands...and now I'm going to touch your hand"	norm / glabellar / pout / palmomental / grasp present		
22.*	One minute hand observation	"Could you put your hands on your knees and relax for a minute while we watch your hands?"	norm / myoclonus / chorea / tremor / other		
23.	Walking	"Could you walk to....and back again for me?"	gait: norm / ataxic / apraxic / cerebellar impairment: norm / mild / mod / sev / wheelchair / bedbound scale: 0 / 1 / 2 / 3 / 4 / 5 / 6		
24.	Heel toe walking	"Can you put one foot in front of the other like this?"	norm / mild / mod / sev / wheelchair / bedbound		
25.	Romberga	"Can you stand with your feet together....and now shut your eyes"	norm / abnormal		
26.*	Neurological exam	Tone/ Power/ Reflexes (esp plantar)	norm tone / pow / ref abnormal tone / pow / ref	norm tone / pow / ref abnormal tone / pow / ref	

Overall assessment

	Yes	No	Fluctuates
Is patient able to cope with task demands?			
Is overall level of attention and concentration satisfactory?			
Is the patient cooperative?			

Overall impression of impairment in this patient was:

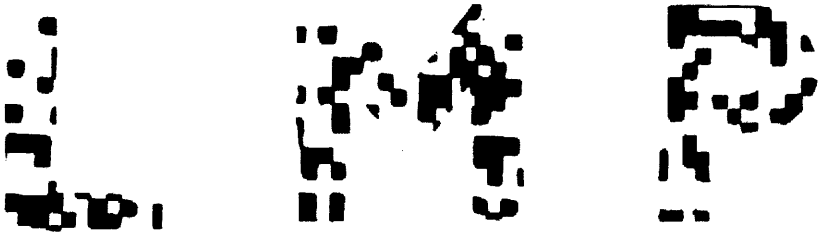
	None	Mild	Moderate	Severe	Cannot assess
Cognitive impairment					
Extrapyramidal impairment					
Pyramidal impairment					
Cerebellar impairment					

Signature		Print name		Date		Video Identification Number	

Appendix N: Cognitive picture tests

a) Line drawings test

b) Fragmented letters task



c) Fragmented objects task

d) Letter cancelling task

B	C	E	A	D	E	C	B
B	E	B	D	C	A	E	A
C	E	D	B	E	C	A	E
E	D	C	B	D	C	E	B
A	E	D	E	A	E	D	A

John Gifford was the kind of person who would go off sailing in his yacht the 'Neva' around the island of Scorba whenever there were signs of chaos at work. He had a thorough knowledge of this area as he had grown up there in his childhood, and this was how he always sought relaxation from the busy routine of office life at Bergess, Challice & Co. He would sit on deck sipping his champagne, as the breeze tangled his normally immaculately combed hair. Gradually his business worries would recede. Dressed in his old, baggy sweater, decrepit suede boots and with his stubbly beard he felt quite the part of an ancient mariner.

Appendix O: Independent Neurologist Score sheet

FORM 7A

PRION-1 TRIAL
INDEPENDENT NEUROLOGIST
NEUROLOGICAL EXAMINATION (VIDEO ASSESSMENT)

COMPLETE DETAILS AND SCORESHEET COMPLETED

Initials: Soundex: / / /
Date of birth: / /
Sex: M ☐ F ☐
Trial visit week/month:
Assessment date: __/__/____

PRION-1 Study Number | | | | | | | |

Video Identification Number

Normal Protocol Severely Affected Protocol

NEUROLOGICAL EXAMINATION (VIDEO ASSESSMENT)

Cognitive Function

No.	Label	Task	Workings (Tick if correct, cross if incorrect, leave blank if not tested)	Number Correct (U if unable to quantify)	Level of function		Example
					Normal	Impaired	
1.	ME	Memory	name / age / married or month	/3			
2.	LC	Letter cancelling	A / B / E	/ 12			
3.	LD	Line drawings	scorpion/ bicycle/ spade/ cow/ violin/ hippo/ ladybird/ pegs/ squirrel/ padlock/ beetle/ trolley/ spanner/ cockerel/ guitar/ camel/ bullet/ lily/ nail/ goal/ door handle/ crab/ skateboard/ basket/ duck/ harp/ kangaroo/ spider/ safety pins/ sheep/ key	/ 10			
4.	RP	Reading passage	John / Richard / Jill dysphasia / poor fluency / neologism	norm / mild / mod / sev			
5.	SP	Spelling	aunt / eye / build / humour / neighbour / autumn	/6			
6.	FL	Fragmented letters	E / N / K L / M / P A / C / W	/3			
7.	FO	Fragmented object	teapot / shoe / chair/ guitar or violin pig/ cake/ boat/ car fish/ boat/ plane/ dog	/4			
8.	CA	Calculation	5+4(9), 7+5(12), 13+9(21), 17+25(42)	/4			
9.	MI	Miming	brush teeth / comb hair / use screwdriver	/3			
10.	CG	Copying gestures	1st / 2nd / 3rd	/3			
11.	FS	Frontal lobe sequen	-	norm / mild / mod / sev			
12.	WC	Words begin with	F / A / S				
13.	PV	Proverbs	strike while the iron / too many cooks	/2			
14.	DS	Digit span	5-8-2, 6-4-3-9, 4-2-7-3-1, 6-1-9-4-7-3, 5-9-1-7-4-2-8	/5			
15.	RC	Recall		norm / mild / mod / sev			
				Total:			

CRF Version 2.4, April 2006

Motor Function

No.	Label	Task	Right	Left	Normal	Impaired	Example
16.	EM	Eye movements	nystagmus / failure of upgaze / other				
17.	FN	Finger-nose testing	norm / mild / mod / sev	norm / mild / mod / sev			
18.	RA	Rapid alternating hand mvmts	norm / mild / mod / sev	norm / mild / mod / sev			
19.	SI	Sequential index finger tapping	norm / mild / mod / sev	norm / mild / mod / sev			
20.	SO	Sequential opposition	norm / mild / mod / sev	norm / mild / mod / sev			
21.	PR	Primitive reflexes	glabellar / pout / palmomental / grasp present				
22.	OM	One minute hand observation	myoclonus / chorea / tremor / other				
23.	WA	Walking	gait: norm / ataxic / apraxic / cerebellar				
			impairment: norm / mild / mod / sev / wheelchair / bedbound				
			scale: 0 / 1 / 2 / 3 / 4 / 5 / 6				
24.	HT	Heel toe walking	norm / mild / mod / sev / wheelchair / bedbound				
25.	RH	Rombergs	-				
26.	NE	Neurological examination	UL tone / pow / ref	UL tone / pow / ref			
		(tick normal, cross abnormal)	LL tone / pow / ref	LL tone / pow / ref			
				Total:			

Overall assessment

	Yes	No	Fluctuates
Is patient able to cope with task demands?			
Is overall level of attention and concentration satisfactory?			
Is the patient cooperative?			

Overall impression of impairment in this patient was:

	None	Mild	Moderate	Severe	Cannot assess
Cognitive impairment					
Extrapyramidal impairment					
Pyramidal impairment					
Cerebellar impairment					

Signature		Print name		Date		Confirm Video Identification Number	

Appendix P: BPRS (Brief Psychiatric Rating Scale)

FORM 8 | PRION-1 TRIAL
BPRS

MRC PRION-1

Date test performed: Day / Month / Year 2 0 0 | Male ○ Female ○ | Initials | Soundex

Date of birth: 1 9 | Inpatient ☐ Outpatient ☐ Home ☐ | Hospital Number

PRION-1 Study Number | Visit month number: 0 ☐ 1 ☐ 2 ☐ 4 ☐ 6 ☐ 9 ☐ 12 ☐ 15 ☐ 18 ☐ 21 ☐ 24 ☐ Extra ☐

BRIEF PSYCHIATRIC RATING SCALE (BPRS)

Each of the 24 symptoms should be rated on the 7-point scale below. **Circle the number** headed by the term that best describes the patient's present condition. If a specific symptom is not rated, mark 'NA' (not assessed).

	not assessed	not present	very mild	mild	moderate	moderately severe	severe	extremely severe	Score
Somatic concern	NA	1	2	3	4	5	6	7	
Anxiety	NA	1	2	3	4	5	6	7	
Depression	NA	1	2	3	4	5	6	7	
Suicidality	NA	1	2	3	4	5	6	7	
Guilt	NA	1	2	3	4	5	6	7	
Hostility	NA	1	2	3	4	5	6	7	
Elated Mood	NA	1	2	3	4	5	6	7	
Grandiosity	NA	1	2	3	4	5	6	7	
Suspiciousness	NA	1	2	3	4	5	6	7	
Hallucinations	NA	1	2	3	4	5	6	7	
Unusual thought content	NA	1	2	3	4	5	6	7	
Bizarre behaviour	NA	1	2	3	4	5	6	7	
Self-neglect	NA	1	2	3	4	5	6	7	
Disorientation	NA	1	2	3	4	5	6	7	
Conceptual disorganisation	NA	1	2	3	4	5	6	7	
Blunted affect	NA	1	2	3	4	5	6	7	
Emotional withdrawal	NA	1	2	3	4	5	6	7	
Motor retardation	NA	1	2	3	4	5	6	7	
Tension	NA	1	2	3	4	5	6	7	
Uncooperativeness	NA	1	2	3	4	5	6	7	
Excitement	NA	1	2	3	4	5	6	7	
Distractibility	NA	1	2	3	4	5	6	7	
Motor hyperactivity	NA	1	2	3	4	5	6	7	
Mannerisms and posturing	NA	1	2	3	4	5	6	7	
								Total Score (sum) =	

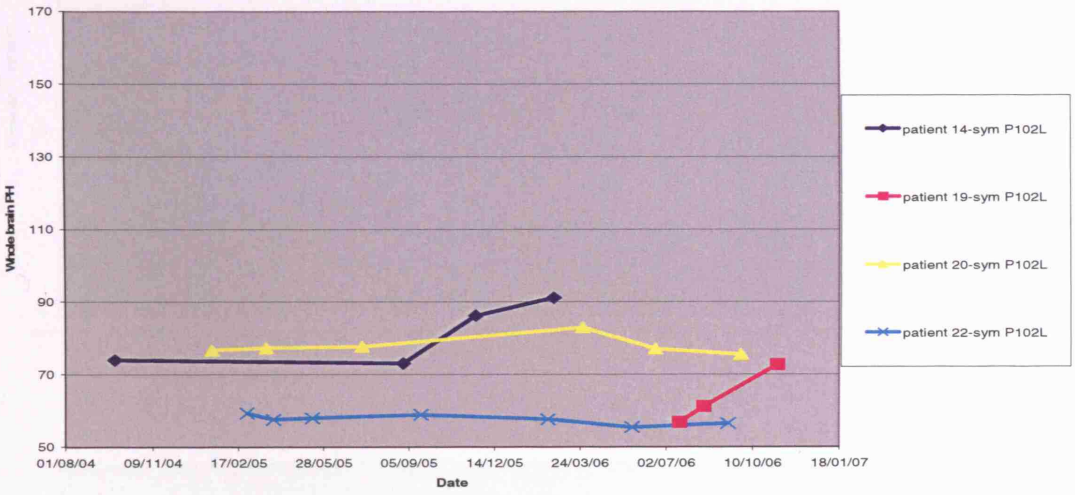
Doctor's signature	Print name	Date

CRF Version 2.0, January 2006

From Ventura, Green, Shaner & Liberman (1993) Training and quality assurance with the brief psychiatric rating scale: "The drift busier" International Journal of Methods in Psychiatric Research.

Appendix Q: Longitudinal changes in MTR histogram measures and ROIs in P102L patients

Whole brain (a-e), white matter (f-j) and grey matter (k-o) MTR histogram measures, and mean ROI MTRs (p-t) either remain steady, plateau after an initial rise or are gradually returning to baseline.



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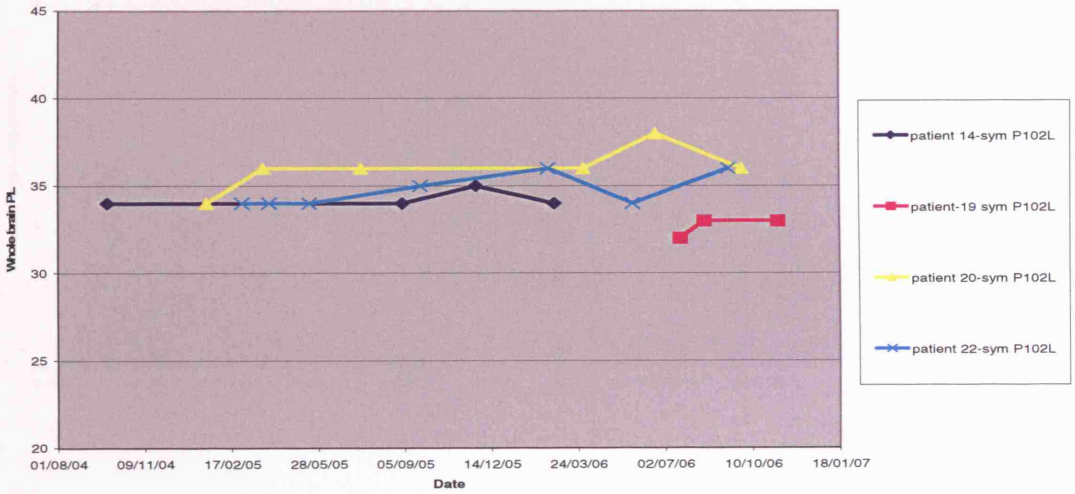

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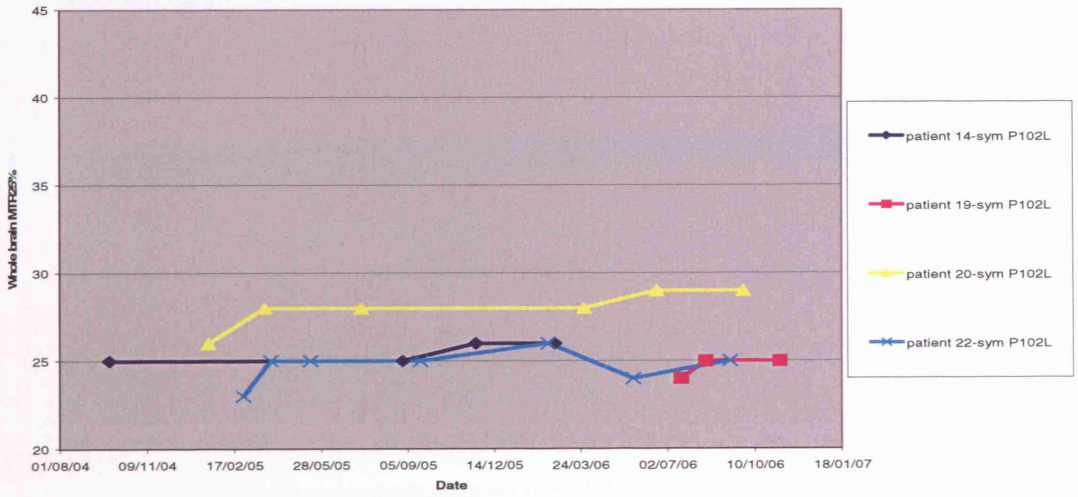

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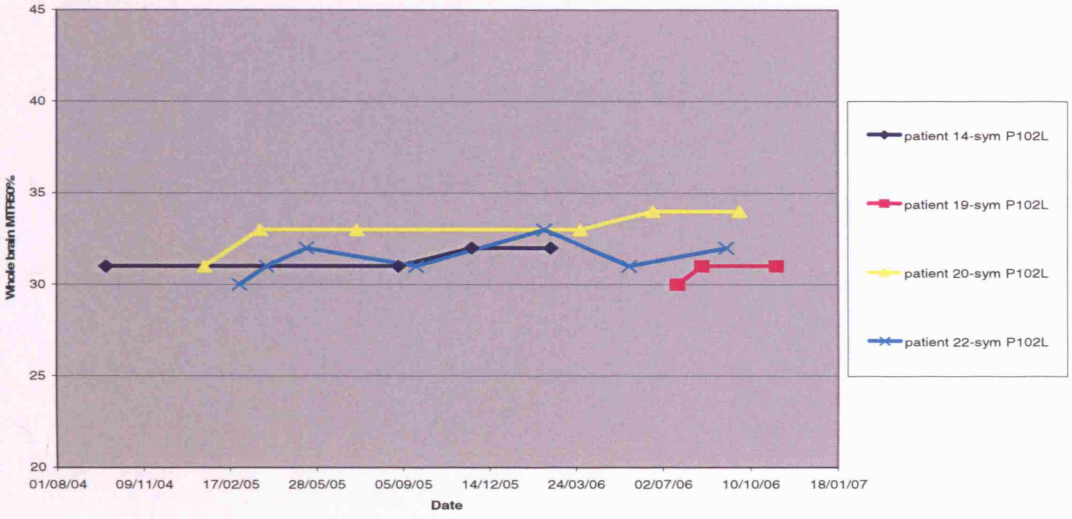
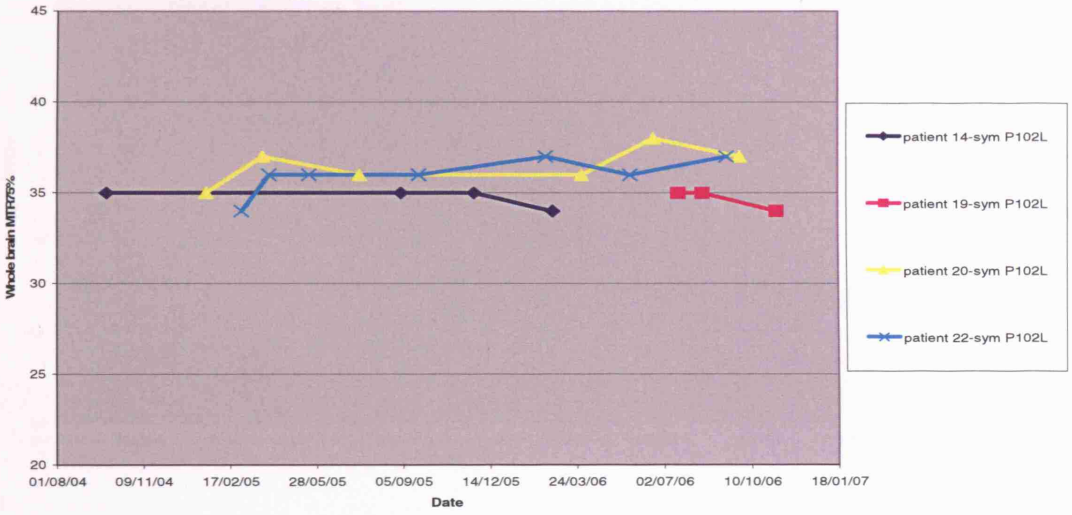

45
40
35
30
25
20
Whole brain MTR50%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

d)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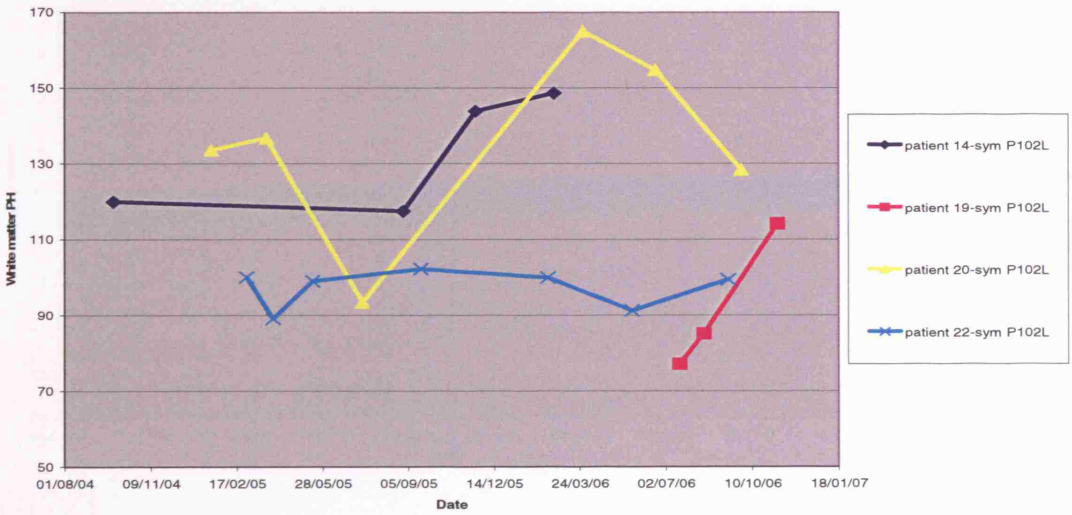
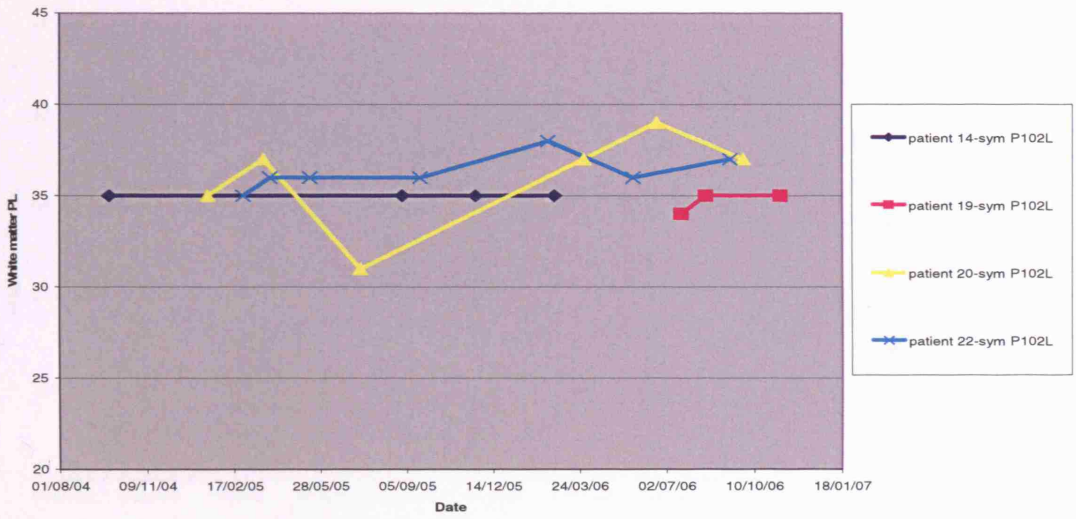
25
20
Whole brain MTR75%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

e)


170
150
130
110
90
70
50
White matter PH
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

f)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40
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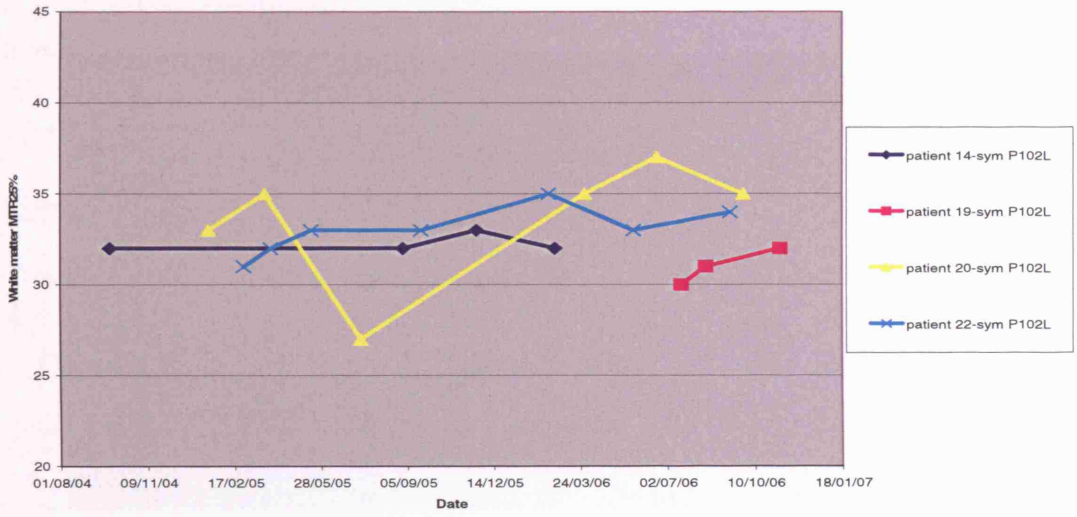
25
20
White matter PL
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

g)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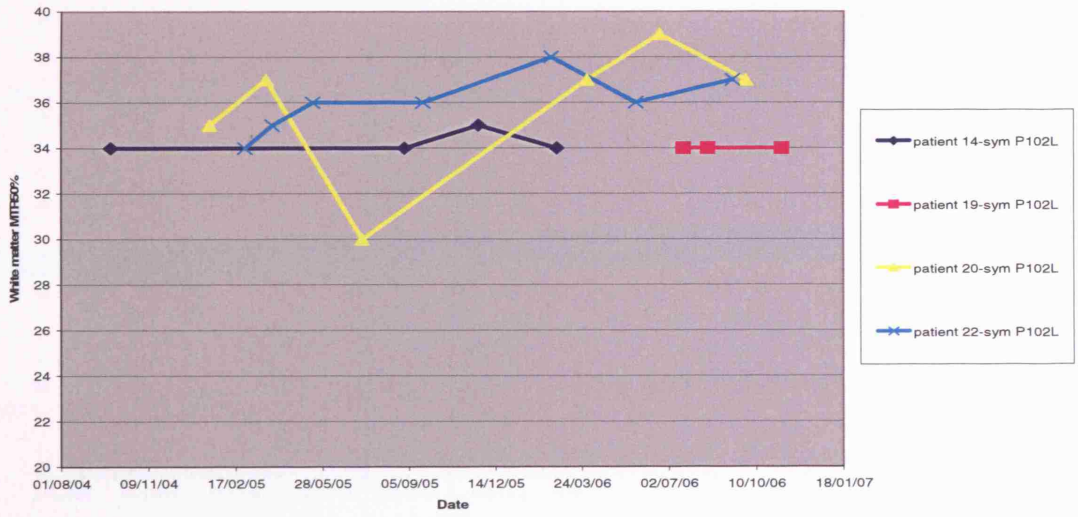
40
35
30
25
20
White matter MTR25%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

h)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38
36
34
32
30
28
26
24
22
20
White matter MTR50%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
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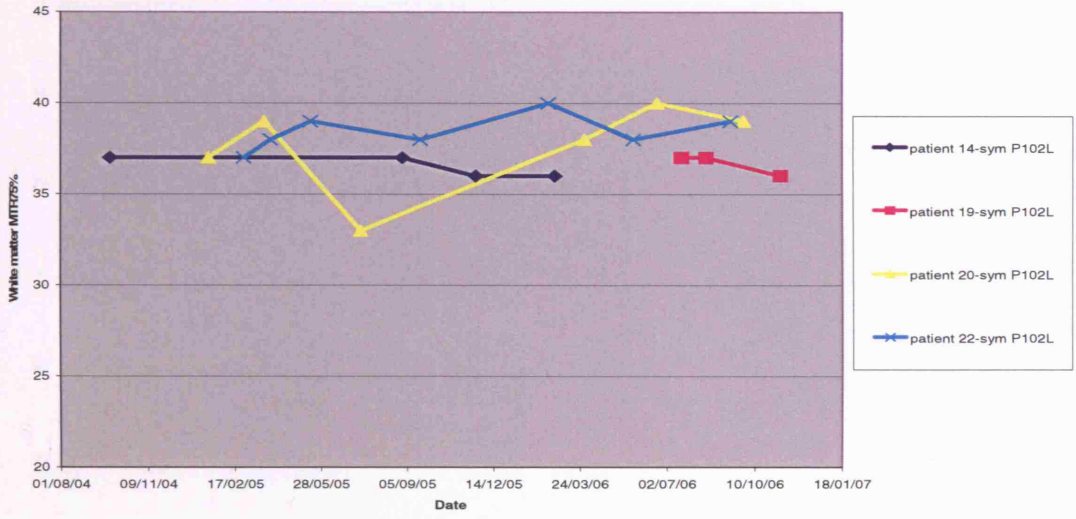
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

i)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40
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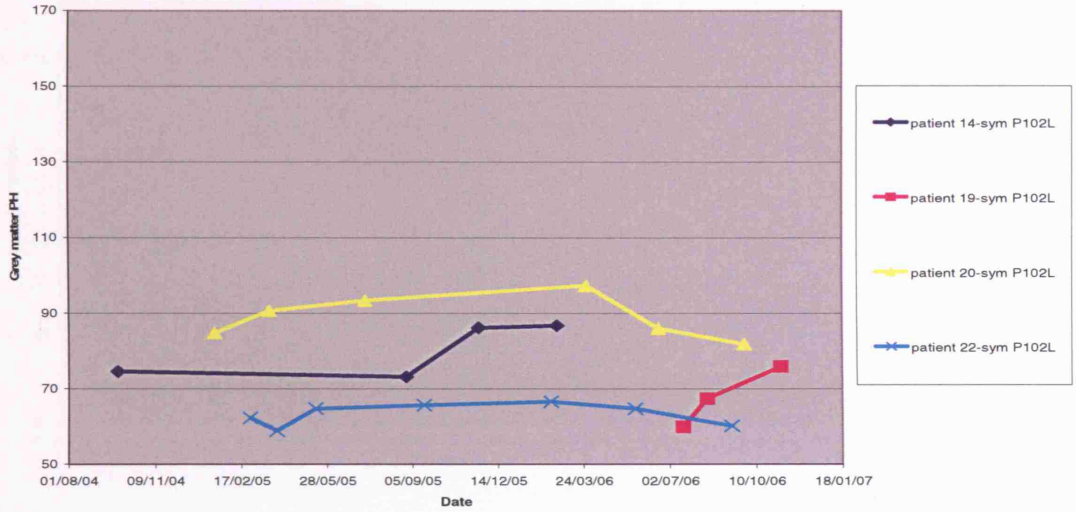
20
White matter MTR/5%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

j)


170
150
130
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70
50
Grey matter PH
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
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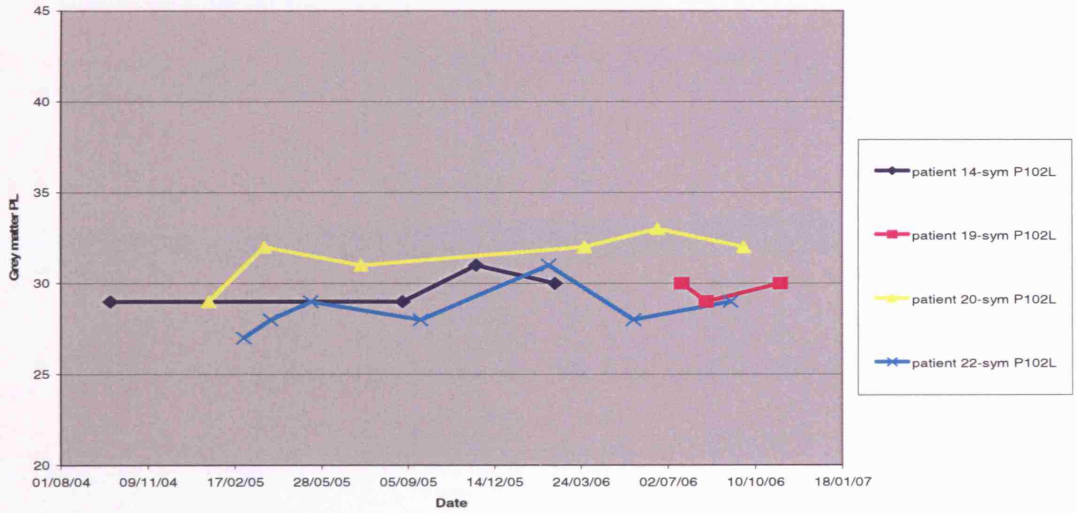
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

k)


45
40
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25
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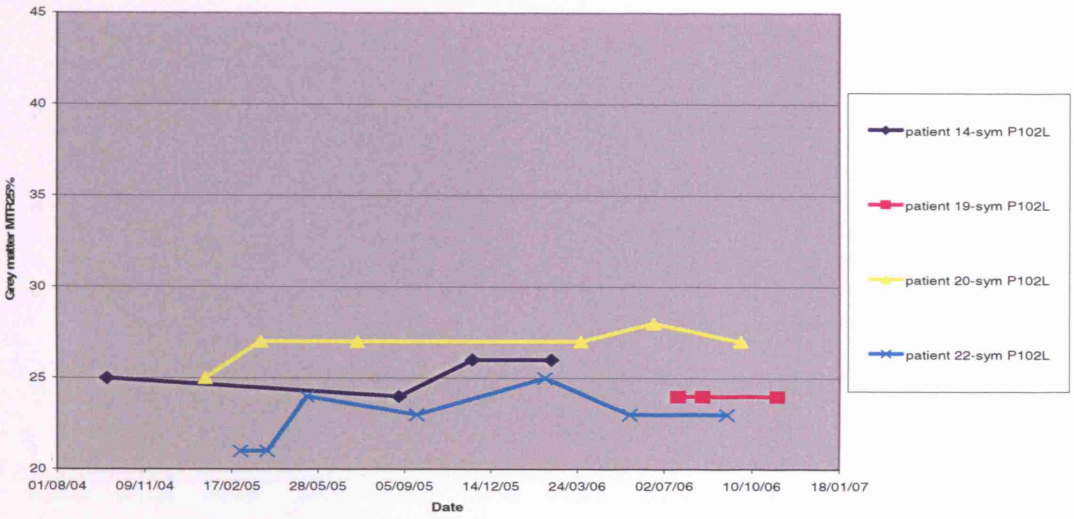
Grey matter PL
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

l)


45
40
35
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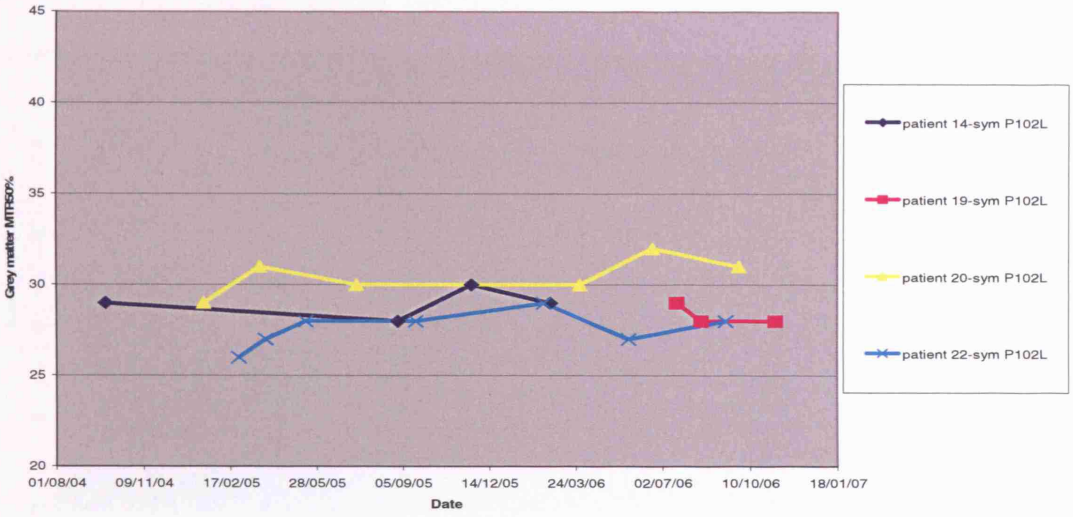
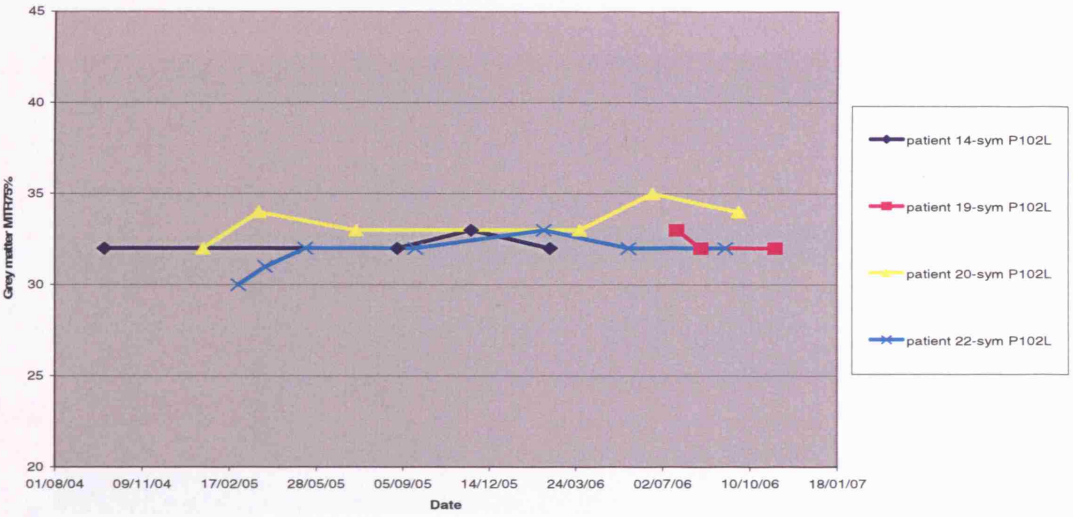
25
20
Grey matter MTR25%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

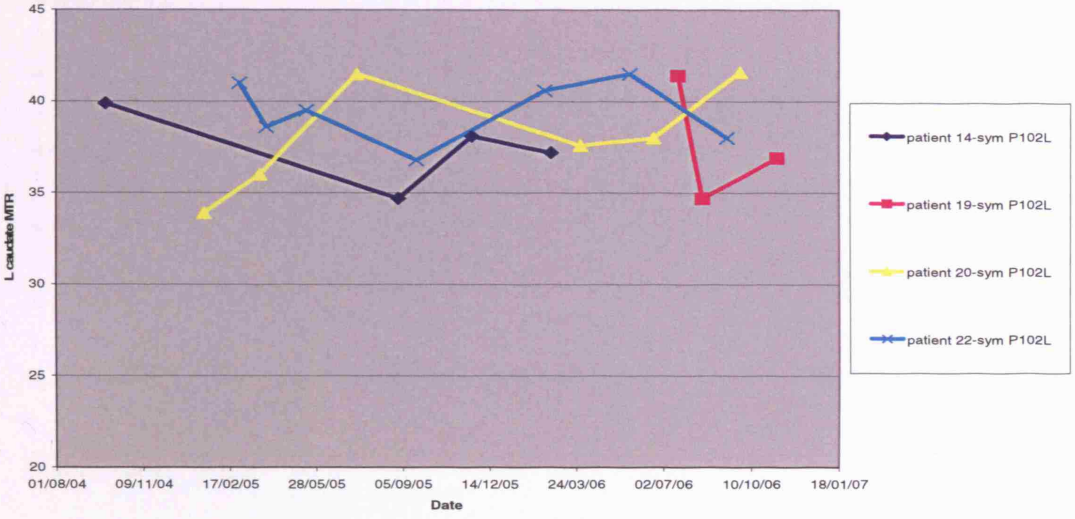
m)


45
40
35
30
25
20
Grey matter MTR50%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

n)


45
40
35
30
25
20
Grey matter MTR75%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

o)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20
L caudate MTR
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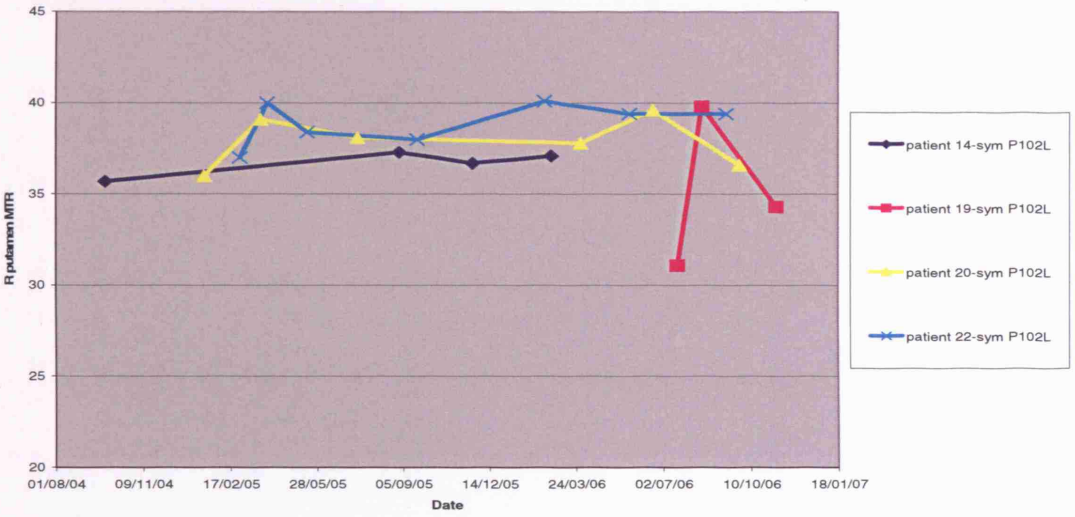
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

p)


45
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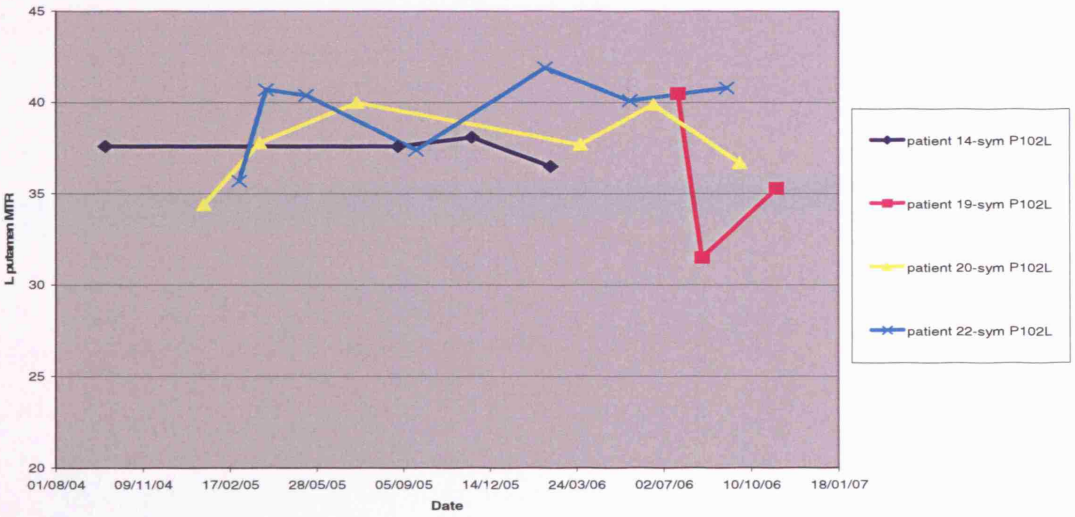
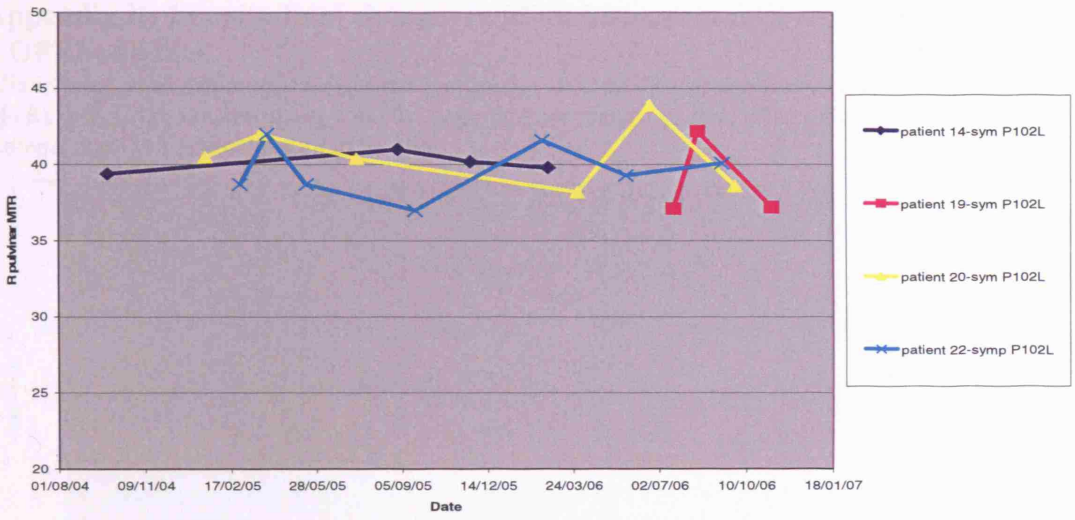
20
R putamen MTR
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

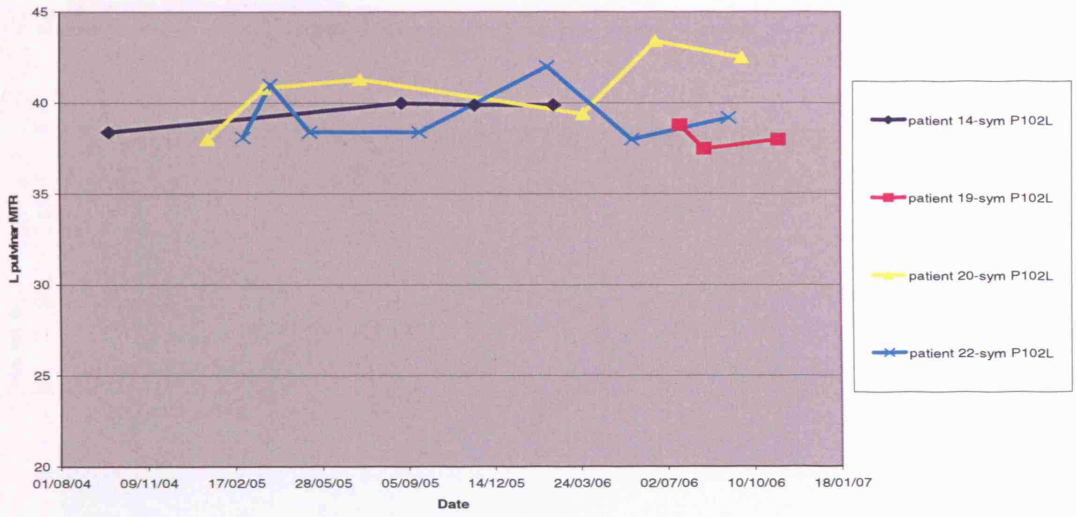
q)


45
40
35
30
25
20
L putamen MTR
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

r)


R pulvinar MTR
50
45
40
35
30
25
20
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-symp P102L

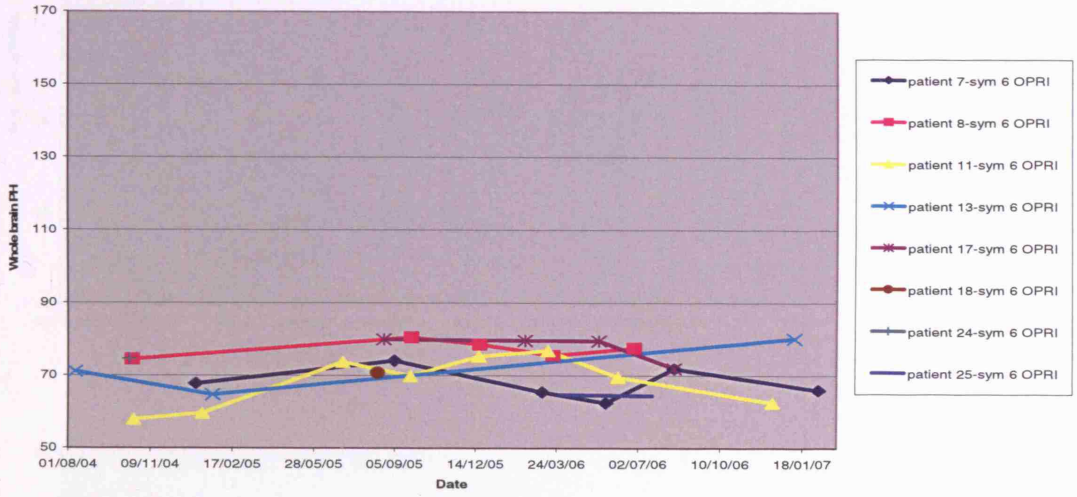
s)


L pulvinar MTR
45
40
35
30
25
20
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 14-sym P102L
patient 19-sym P102L
patient 20-sym P102L
patient 22-sym P102L

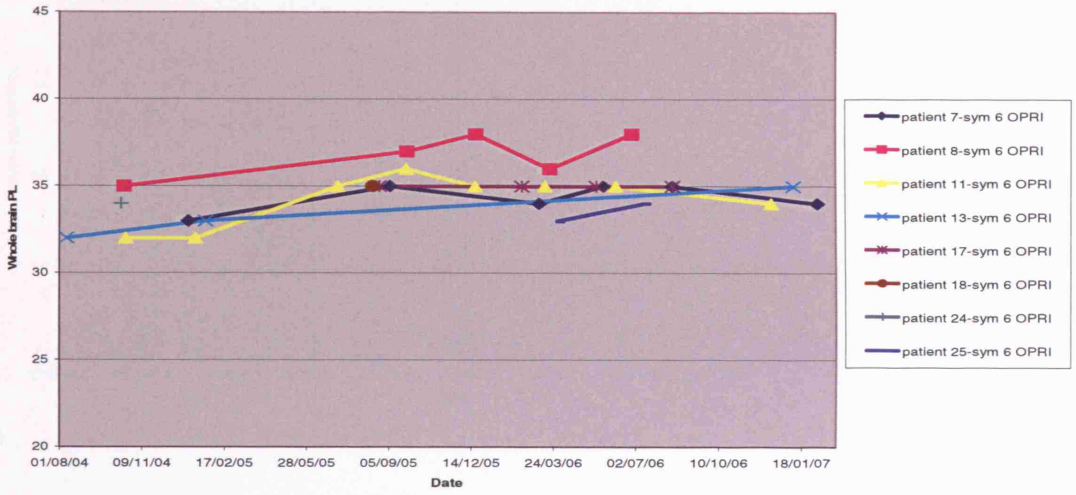
t)

Appendix R: Longitudinal changes in MTR histogram measures and ROIs in 6 OPRI patients

Whole brain (a-e), white matter (f-j) and grey matter (k-o) MTR histogram measures, and mean ROI MTRs (p-t) either remain steady from the beginning, or reach a plateau after an initial rise. In some patients there is a gradual return to baseline.



a)



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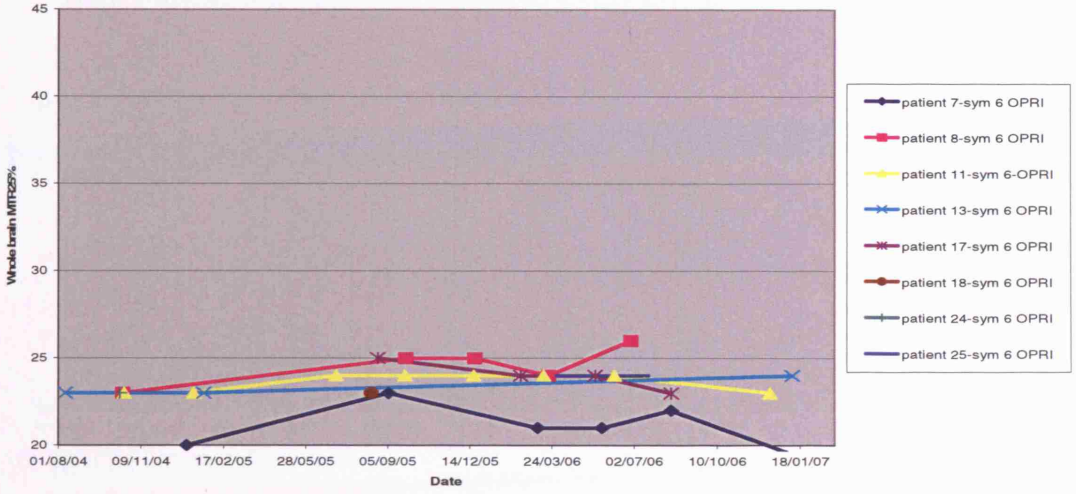
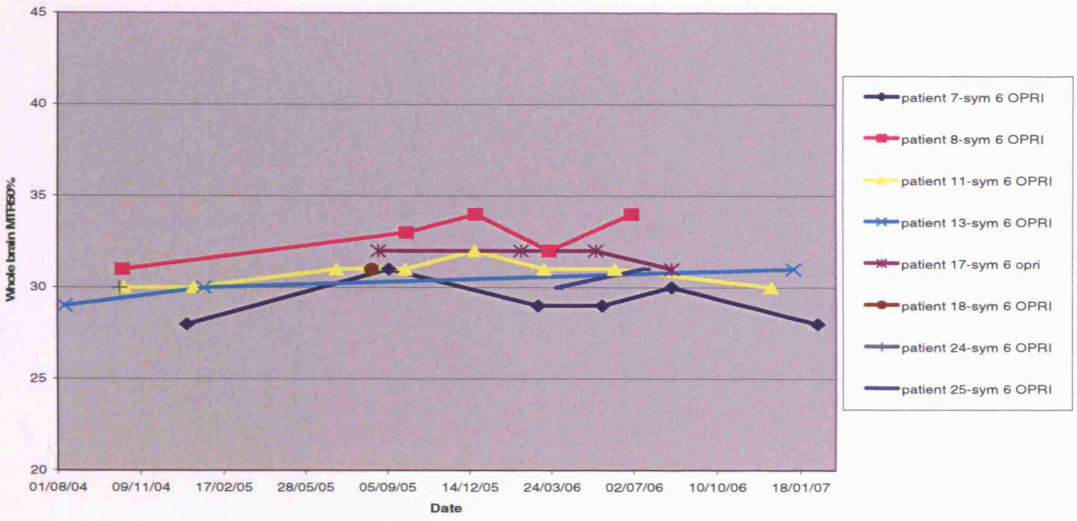
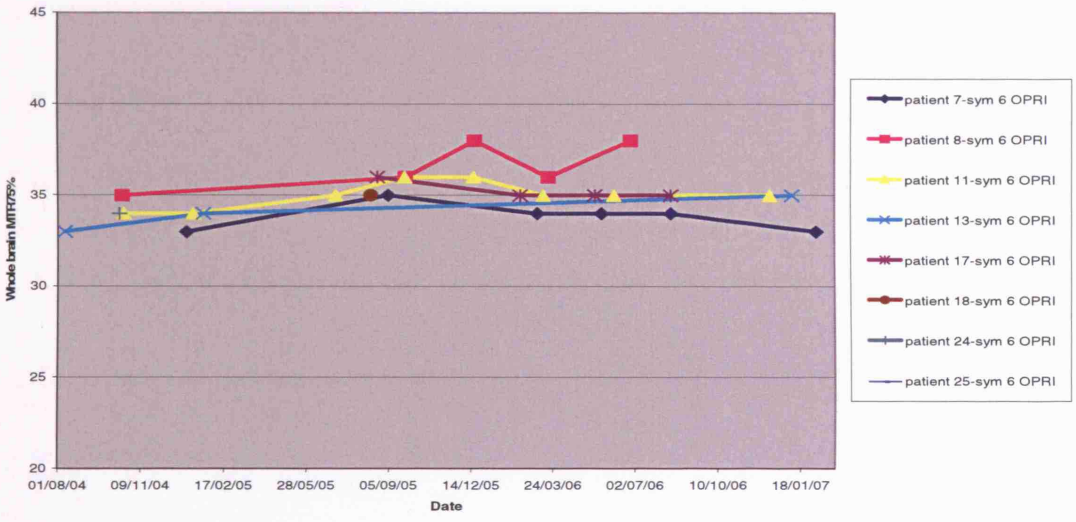


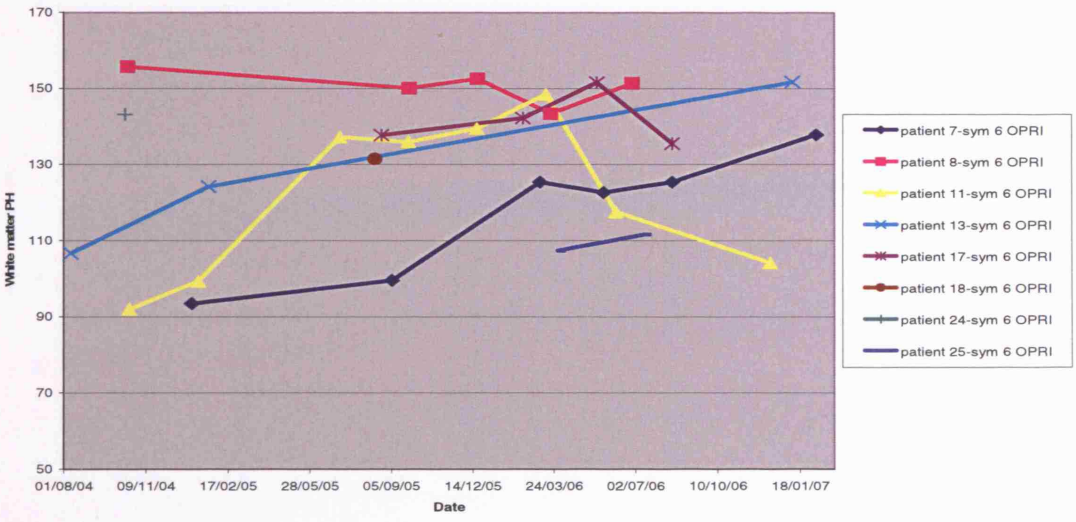
c)


45
40
35
30
25
20
Whole brain MTR60%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 opri
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

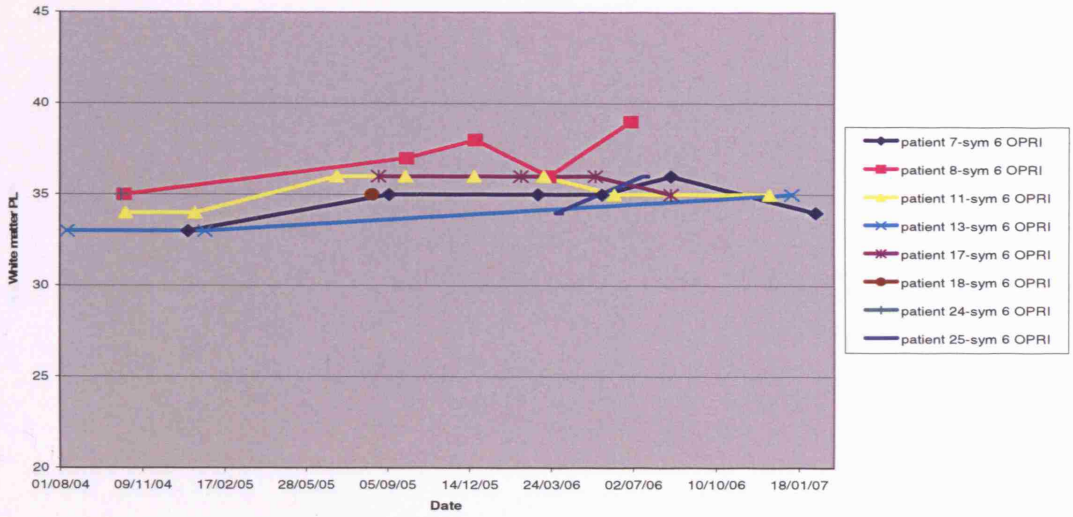
d)


45
40
35
30
25
20
Whole brain MTR75%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

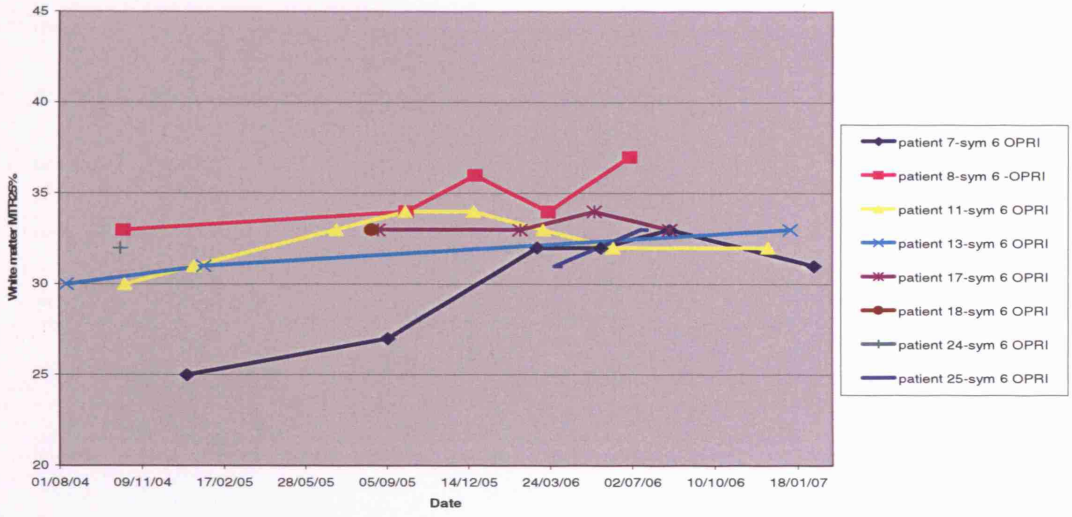
e)


170
150
130
110
90
70
50
White matter PH
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

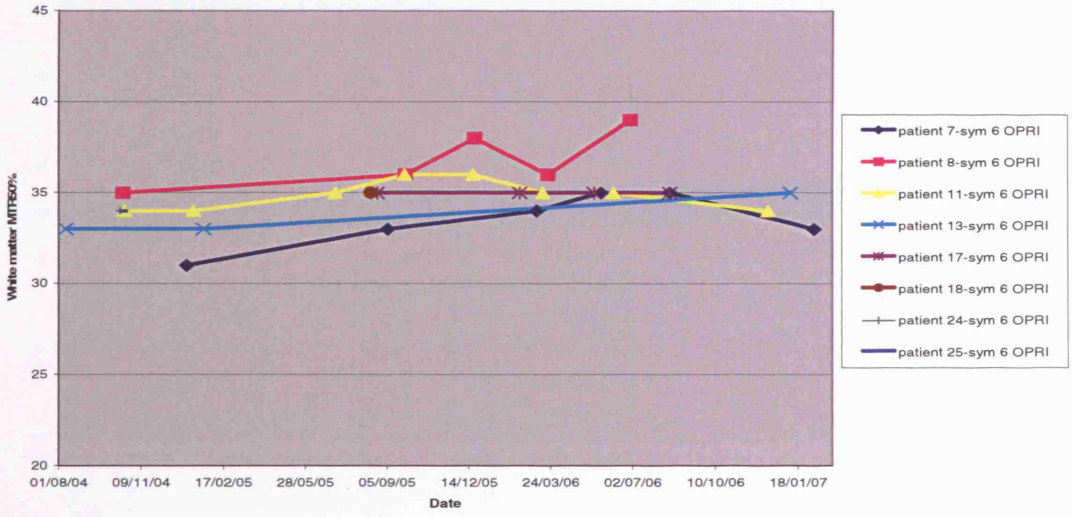
f)


45
40
35
30
25
20
White matter PL
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

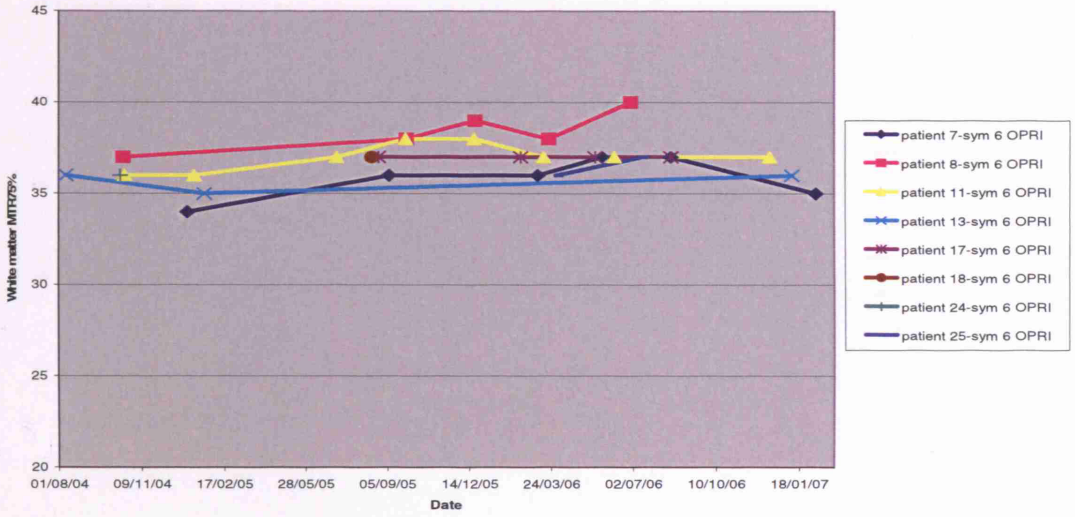
g)


45
40
35
30
25
20
White matter MTP25%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 -OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

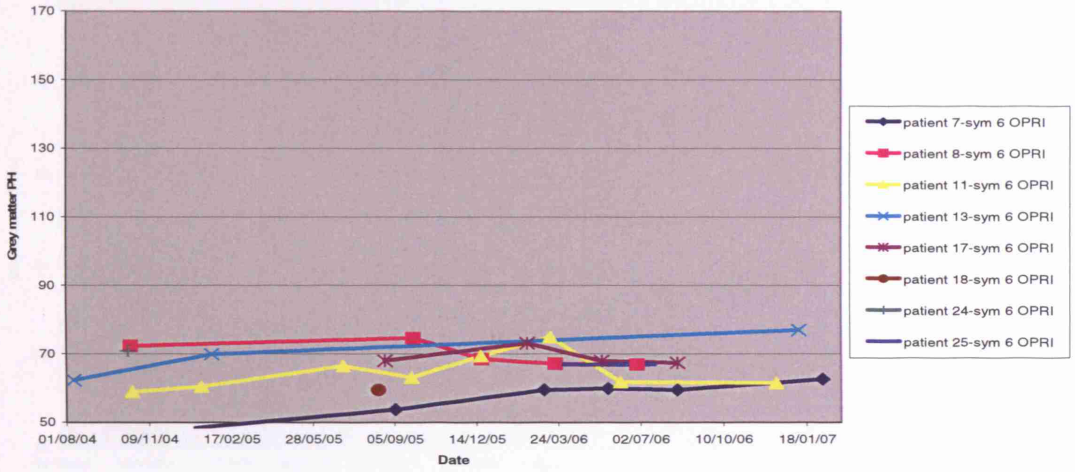
h)


45
40
35
30
25
20
White matter MTP60%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

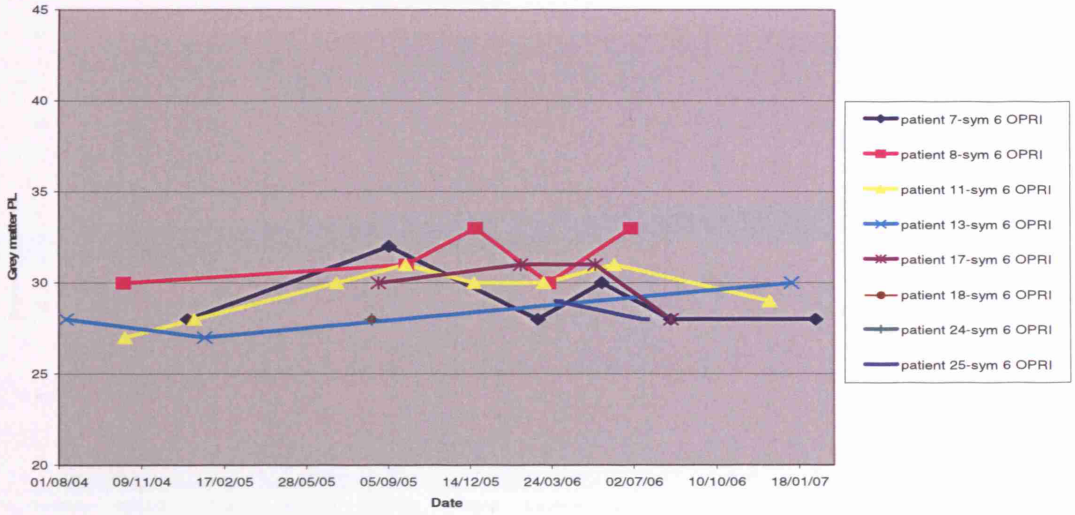
i)


45
40
35
30
25
20
White matter MTR/%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

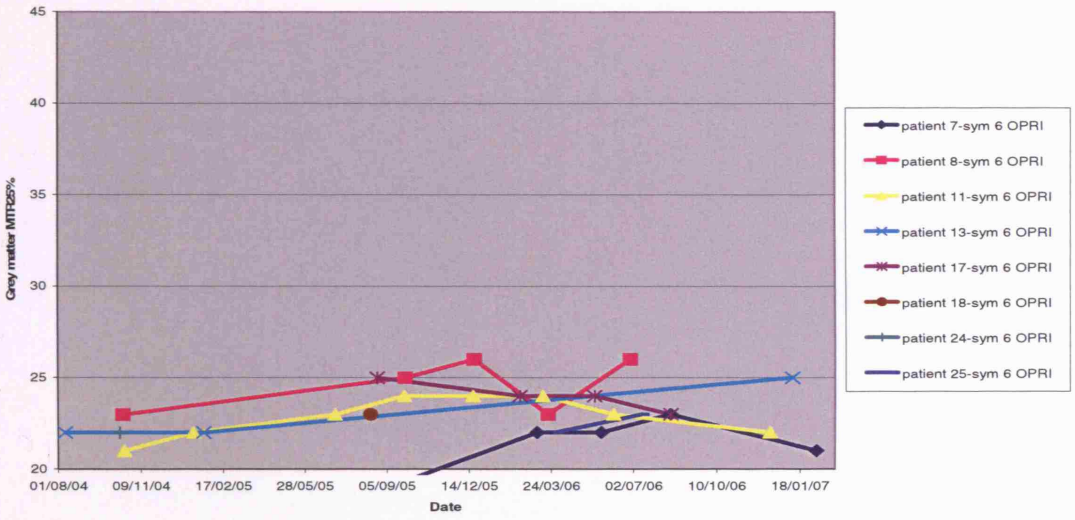
j)


170
150
130
110
90
70
50
Grey matter PH
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

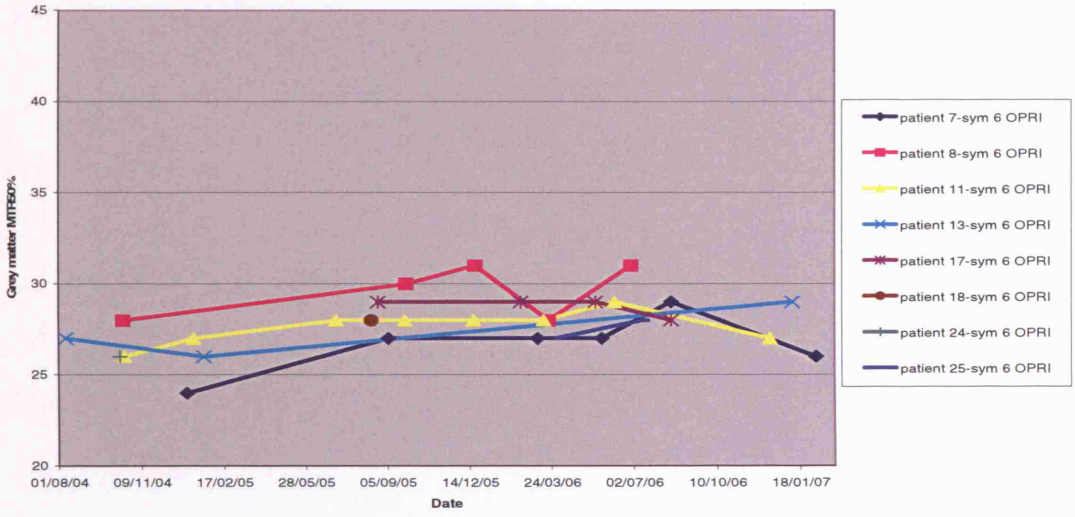
k)


45
40
35
30
25
20
Grey matter PL
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

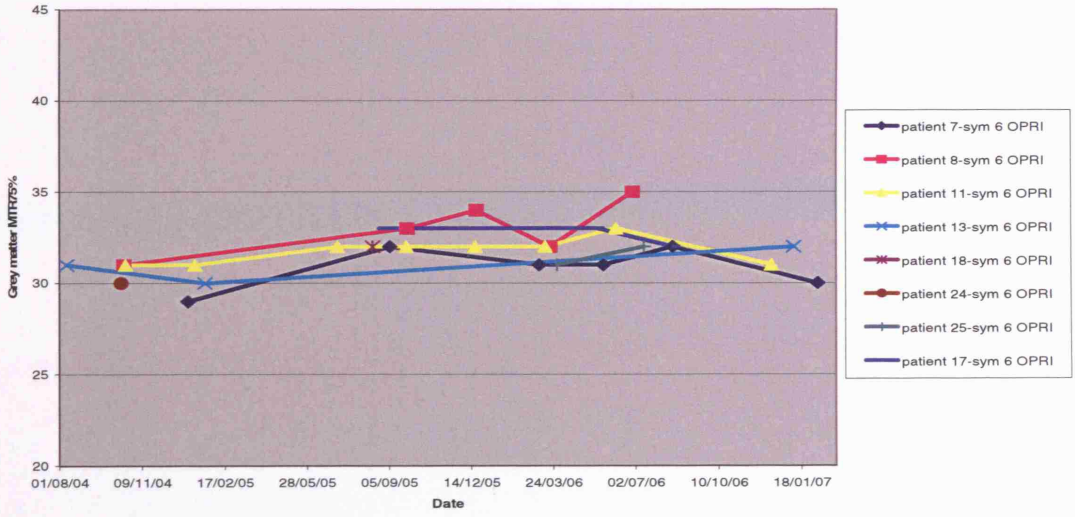
l)


45
40
35
30
25
20
Grey matter MTR25%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

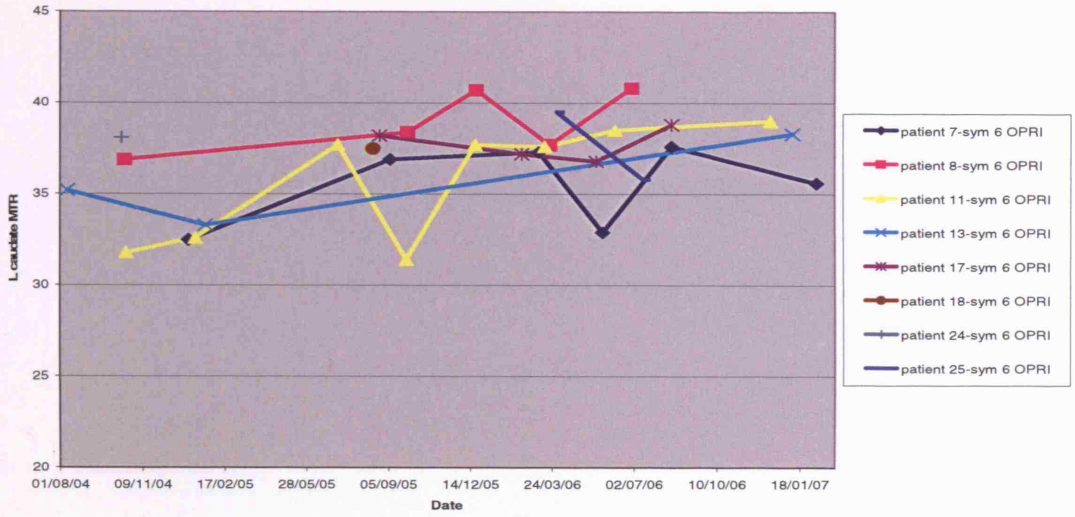
m)


45
40
35
30
25
20
Grey matter MTR50%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

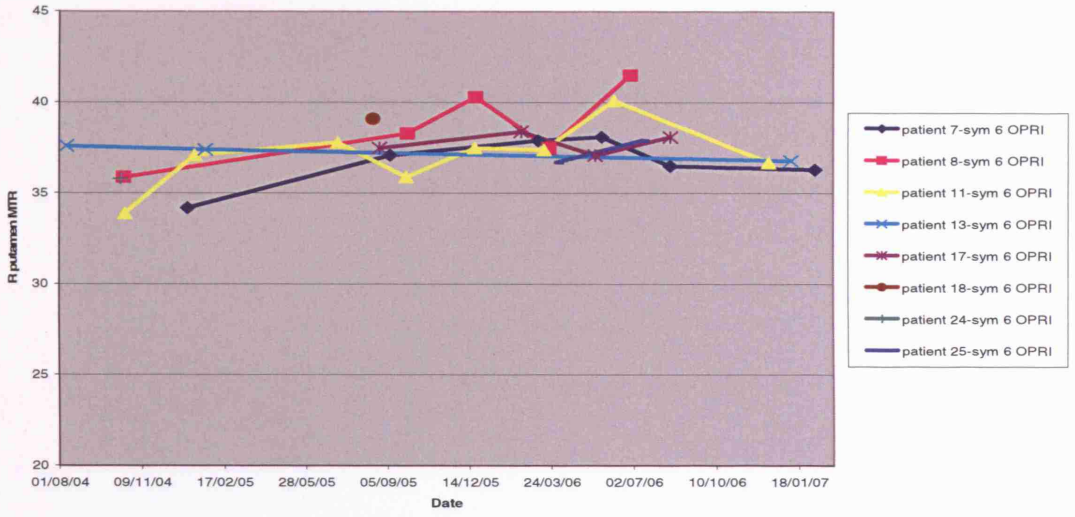
n)


45
40
35
30
25
20
Grey matter MTR75%
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI
patient 17-sym 6 OPRI

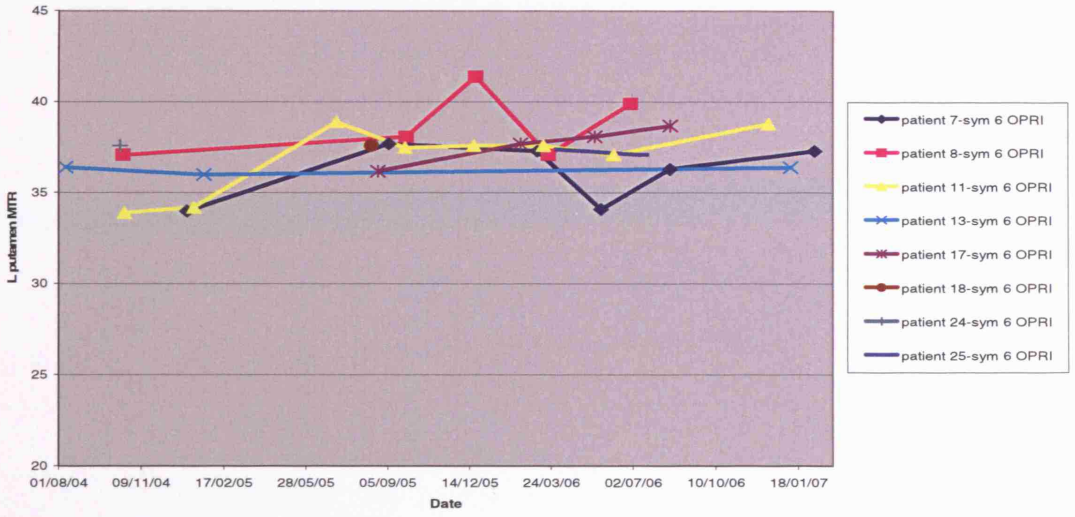
o)


L caudate MTR
45
40
35
30
25
20
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

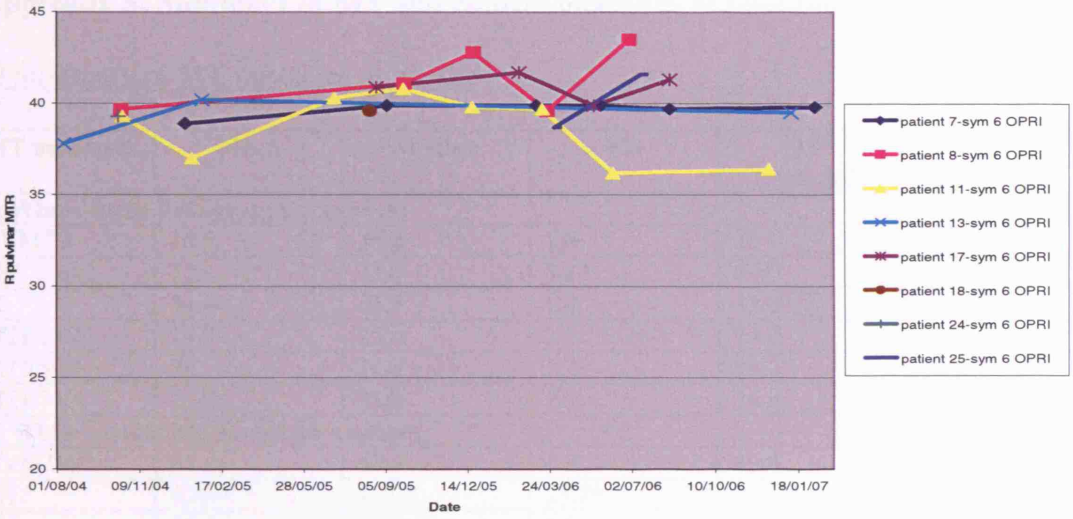
p)


R putamen MTR
45
40
35
30
25
20
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

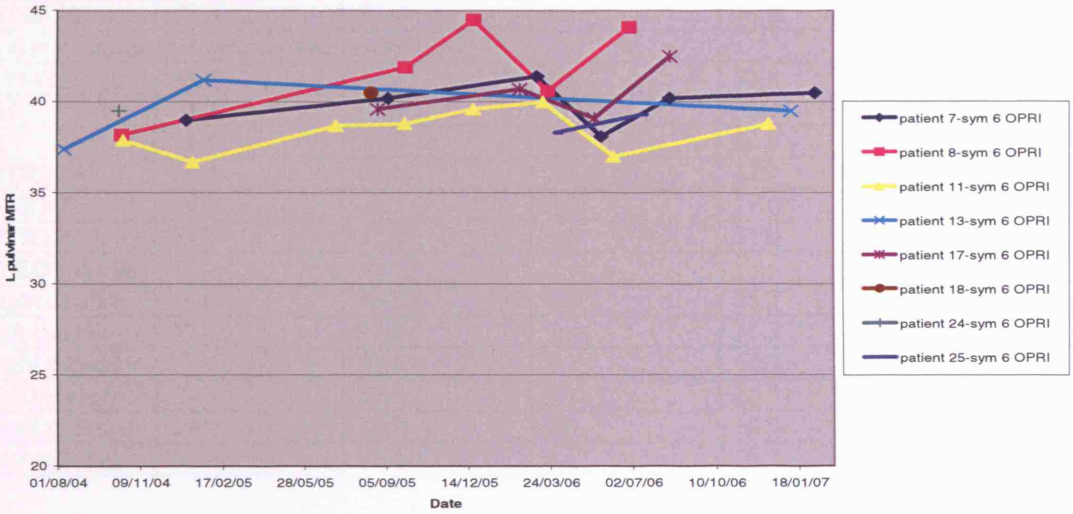
q)


L putamen MTR
45
40
35
30
25
20
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

r)


45
40
35
30
25
20
R pulvinar MTR
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

s)


45
40
35
30
25
20
L pulvinar MTR
01/08/04
09/11/04
17/02/05
28/05/05
05/09/05
14/12/05
24/03/06
02/07/06
10/10/06
18/01/07
Date
patient 7-sym 6 OPRI
patient 8-sym 6 OPRI
patient 11-sym 6 OPRI
patient 13-sym 6 OPRI
patient 17-sym 6 OPRI
patient 18-sym 6 OPRI
patient 24-sym 6 OPRI
patient 25-sym 6 OPRI

t)

Appendix S: Summary of MT and clinical measures at baseline

a) Summary of MT measures at baseline

MT measures	Mean	Median	SD	25th percentile	75th percentile
a) Whole brain histogram parameters					
AVMTR	36.5	36.4	1.6	35.4	37.2
PH	72.4	73.6	8.8*	67.0	80.9
PL	34.4	34.5	1.7	33.0	35.0
MTR25%	24.8	25.0	2.5	23.0	26.0
MTR50%	31.1	31.0	1.7	30.0	32.2
MTR75%	35.0	35.0	1.5	34.0	36.0
b) White matter histogram parameters					
AVMTR	41.4	41.4	1.9	40.4	42.3
PH	129.4	132.6	25.7*	106.7	150.8
PL	35.3	35.0	1.7	34.0	36.0
MTR25%	32.5	33.0	2.5	31.0	34.0
MTR50%	34.9	35.0	1.9	34.0	36.0
MTR75%	37.0	37.0	1.5	36.0	38.0
c) Grey matter histogram parameters					
AVMTR	35.2	35.5	1.8	33.8	35.9
PH	73.9	71.9	11.9*	62.3	84.6
PL	29.5	29.0	1.8	28.0	31.0
MTR25%	24.2	25.0	2.6	22.0	25.2
MTR50%	28.4	28.5	2.0	27.0	29.0
MTR75%	31.9	32.0	1.5	31.0	33.0
d) ROI MTRs					
Right caudate	36.6	36.7	2.4	35.3	38.0
Left caudate	37.5	37.8	2.6	36.3	39.3
Right putamen	37.0	37.1	2.3	35.7	38.4
Left putamen	37.6	37.5	2.1	36.3	39.0
Right pulvinar	39.9	39.7	2.0	38.7	41.0
Left pulvinar	39.3	39.1	2.1	38.2	40.3

* Whole brain, white matter, and grey matter PH lower in symptomatic patients with MMSE<25 or when MMSE unrecordable in severely affected cases, than in asymptomatic and symptomatic patients with MMSE>25, causing higher PH SD at baseline

Key: Average MTR (AVMTR), Peak Height (PH), Peak Location (PL), MTR at 25th percentile (MTR25%), MTR at 50th percentile (MTR50%), and MTR at 75th percentile (MTR75%)

b) Summary of clinical measures at baseline

Clinical measures	Mean	Median	SD	25th percentile	75th percentile
MMSE	22.9	23.0	6.5*	19.0	29.0
ADL	17.4	18.5	3.7	16.8	20.0
ADAS-COG	14.1	12.5	11.4*	6.0	20.0
CDR	4.7	4.0	4.1*	0.8	7.25
CGIS	3.2	4.0	1.4	1.8	4.0
GCS	14.9	15.0	0.3	15.0	15.0
Rankin	2.2	3.0	1.4	1.50	3.0
BPRS	33.7	33.0	6.6*	29.0	37.0
Cognitive impairment	1.9	2.00	1.0	1.0	3.0
Extrapyramidal	0.6	0.0	1.3	0.0	0.3

impairment					
Pyramidal impairment	0.6	0.0	1.1	0.0	1.0
Cerebellar impairment	1.1	1.0	1.3	0.0	2.0

*Higher SDs in MMSE, ADAS-COG, CDR and BPRS due to a combination of poorer ratings in symptomatic patients, and normal or near-normal ratings in asymptomatic patients at baseline

Appendix T: Tables showing slopes of change over time in MTR histogram and ROI parameters

a) Slopes of change over time in whole brain histogram parameters

	AVMTR	PH	PL	MTR25%	MTR50%	MTR75%
Pt 1	-0.04	-0.24	-0.03	-0.07	-0.06	-0.03
Pt 5	0.02	-0.002	0.01	0.01	0.01	0.03
Pt 6	-0.003	0.008	-0.01	0.000	0.000	-0.01
Pt 7♦	-0.001	-0.03	0.01	-0.009	-0.002	-0.002
Pt 8♦	0.03	0.03	0.03	0.03	0.03	0.03
Pt 9	-0.006	-0.05	0.000	0.000	0.000	0.000
Pt 10♦	0.01	0.35	0.07	0.000	0.000	0.000
Pt 11♦	0.009	0.16	0.04	0.01	0.01	0.02
Pt 12	-0.06	0.25	0.000	-0.10•	0.000	0.000
Pt 13♦	0.01	0.10	0.02	0.008	0.01	0.01
Pt 14♦	-0.001	0.21	0.005	0.01	0.01	-0.009
Pt 15	-0.008	0.05	-0.02	-0.02	-0.02	-0.002
Pt 17	-0.02	-0.13	0.000	-0.08	0.00	-0.02
Pt 19	-0.007	0.25	0.05	0.05	0.05	-0.07
Pt 20	0.02	0.006	0.02	0.02	0.02	0.02
Pt 21	-0.009	-0.24	-0.01	-0.05	-0.01	0.000
Pt 22	0.01	-0.03	0.02	0.009	0.02	0.02
Pt 23	-0.03	-0.09	-0.03	-0.03	-0.03	-0.03
Pt 25	0.04	-0.02	0.07	0.000	0.07	0.07
Pt 26	0.002	0.10	0.02	0.02	0.000	0.000
Mean	-0.002	0.03	0.01	-0.009	0.006	0.000
SD	0.02	0.16	0.03	0.04	0.03	0.03
Median	-0.001	0.006	0.01	0.000	0.000	0.000
25th percentile	-0.008	-0.05	-0.01	-0.03	-0.001	-0.01
75th percentile	0.01	0.14	0.03	0.01	0.01	0.02
p values from one-sample t-test	0.70	0.35	0.06	0.30	0.35	0.92

♦ Patients who had scans under GA

• Truncated slope

b) Slopes of change over time in white matter histogram parameters

	AVMTR	PH	PL	MTR25%	MTR50%	MTR75%
Pt 1	-0.03	-0.31	-0.03	-0.05	-0.03	-0.01
Pt 5	0.02	-0.05	0.01	0.01	0.03	0.03
Pt 6	-0.006	-0.02	0.000	-0.01	-0.01	0.000
Pt 7♦	0.04	0.43	0.01	0.07	0.03	0.02
Pt 8♦	0.03	-0.08	0.04	0.04	0.03	0.03
Pt 9	-0.002	0.04	0.000	0.000	0.000	0.000
Pt 10♦	0.01	0.71	0.000	0.000	0.000	0.000
Pt 11♦	0.02	0.39	0.02	0.03	0.01	0.02
Pt 12	-0.04	1.00	0.000	0.13	0.000	-0.05
Pt 13♦	0.01	0.33	0.02	0.02	0.02	0.003

Pt 14♦	-0.002	0.38	0.000	0.005	0.005	-0.01
Pt 15	-0.01	0.05	-0.02	-0.006	-0.01	-0.01
Pt 17	-0.006	0.05	-0.02	0.007	0.000	0.000
Pt 19	0.02	1.00	0.05	0.11	0.000	-0.05
Pt 20	0.04	0.24	0.04	0.04	0.04	0.03
Pt 21	-0.004	-0.62	-0.01	-0.02	0.008	0.000
Pt 22	0.02	0.008	0.02	0.03	0.03	0.02
Pt 23	-0.04	-0.65	-0.06	-0.06	-0.04	-0.03
Pt 25	0.04•	0.28	0.13	0.13	0.04	0.05
Pt 26	0.002	0.22	0.000	0.000	0.000	0.000
Mean	0.006	0.17	0.009	0.02	0.007	0.000
SD	0.02	0.44	0.04	0.01	0.02	0.03
Median	0.007	0.14	0.000	0.05	0.002	0.000
25th percentile	-0.006	-0.04	-0.01	-0.004	0.000	-0.01
75th percentile	0.02	0.38	0.02	0.04	0.03	0.02
p values from one-sample t-test	0.31	0.10	0.26	0.054	0.15	0.88

♦ Patients who had scans under GA

• Truncated slope

c) Slopes of change over time in grey matter histogram parameters

	AVMTR	PH	PL	MTR25%	MTR50%	MTR75%
Pt 1	-0.03	0.007	-0.03	-0.04	-0.03	-0.05
Pt 5	0.02	-0.06	0.01	0.01	0.01	0.01
Pt 6	0.000	-0.05	0.005	0.005	-0.01	0.005
Pt 7♦	0.02	0.13	-0.01	0.05	0.02	0.01
Pt 8♦	0.02	-0.07	0.03	0.02	0.02	0.04
Pt 9	-0.008	-0.04	0.02	-0.02	-0.02	0.000
Pt 10♦	0.01	0.31	0.07	0.000	0.000	0.000
Pt 11♦	0.02	0.09	0.04	0.02	0.02	0.02
Pt 12	-0.05	0.40•	-0.10	0.000	-0.05	-0.05
Pt 13♦	0.02	0.10	0.02	0.03	0.02	0.01
Pt 14♦	0.004	0.17	0.02	0.01	0.004	0.005
Pt 15	-0.005	0.03	-0.003	-0.006	-0.01	-0.01
Pt 17	-0.02	-0.02	-0.03	-0.04	-0.02	-0.02
Pt 19	-0.03	0.30	0.02	0.000	-0.05	-0.05
Pt 20	0.02	-0.03	0.03	0.02	0.02	0.02
Pt 21	-0.007	-0.11	-0.02	-0.02	-0.006	0.008
Pt 22	0.02	0.01	0.02	0.02	0.01	0.02
Pt 23	-0.03	-0.17	-0.06	-0.03	-0.03	0.000
Pt 25	0.05	-0.004	-0.07	0.07	0.05•	0.07
Pt 26	0.006	0.12	0.02	0.02	0.02	0.000
Mean	0.000	0.05	-0.002	0.006	-0.000	0.001
SD	0.02	0.15	0.04	0.03	0.03	0.03
Median	0.004	0.009	0.02	0.009	0.002	0.005
25th percentile	-0.02	-0.05	-0.03	-0.02	-0.02	-0.001
75th percentile	0.02	0.13	0.02	0.02	0.02	0.02

p values from one-sample t-test	0.88	0.11	0.86	0.34	0.90	0.82

♦ Patients who had scans under GA

• Truncated slope

d) Slopes of change over time in mean ROI MTRs

	Right caudate	**Left caudate**	**Right putamen**	**Left putamen**	**Right pulvinar**	**Left pulvinar**
Pt 1	-0.12	-0.01	-0.05	-0.03	-0.007	0.09
Pt 5	0.004	-0.02	0.009	0.03	0.03	0.02
Pt 6	0.000	-0.01	-0.005	-0.01	0.000	-0.007
Pt 7♦	0.04	0.02	0.02	0.02	0.007	0.009
Pt 8♦	0.05	0.04	0.05	0.03	0.03	0.06
Pt 9	0.06	0.04	0.12	-0.06	-0.01	-0.04
Pt 10♦	0.15	0.18	0.10	0.02	-0.04	0.02
Pt 11♦	0.08	0.08	0.04	0.05	-0.01	0.02
Pt 12	-0.20	0.000	-0.10	-0.10	-0.03	-0.05
Pt 13♦	0.03	0.03	-0.006	0.001	0.007	0.007
Pt 14♦	0.05	-0.04	0.02	-0.008	0.009	0.02
Pt 15	-0.002	-0.01	-0.02	0.006	-0.006	0.008
Pt 17	0.005	0.003	0.005	0.05	-0.002	0.04
Pt 19	-0.10	-0.17	0.05	-0.08	-0.09	-0.03
Pt 20	0.03	0.05	0.004	0.02	-0.01	0.04
Pt 21	0.02	0.04	-0.02	-0.02	-0.04	-0.002
Pt 22	-0.002	0.000	0.02	0.03	0.007	0.003
Pt 23	-0.05	0.000	-0.02	-0.06	0.000	-0.07
Pt 25	-0.17	-0.15	0.08	-0.02	0.10	0.07
Pt 26	0.04	0.04	0.06	0.002	0.03	0.04
Mean	-0.004	0.006	0.02	-0.006	-0.001	0.01
SD	0.09	0.07	0.05	0.04	0.04	0.04
Median	0.01	0.001	0.01	0.001	-0.001	0.01
25th percentile	-0.04	-0.01	-0.01	-0.02	-0.01	-0.005
75th percentile	0.05	0.04	0.05	0.02	0.009	0.04
p values from one-sample t-test	0.85	0.72	0.14	0.49	0.90	0.18

♦ Patients who had scans under GA

Appendix U: Tables showing slopes of change over time in MMSE, ADAS-COG, GCS, BPRS, and videoed cognitive and motor scores

a) Slopes of change over time in MMSE, ADAS-COG, GCS and BPRS

	MMSE	ADAS-COG	GCS	BPRS
Pt 1	-0.06	0.24	*	0.21
Pt 5	-0.01	-0.01	*	0.000
Pt 6	-0.02	-0.12	*	-0.01
Pt 7	-0.07	0.20	-0.01	-0.04
Pt 8	0.000	0.000	-0.01	0.00
Pt 9	-0.02	0.000	*	0.000
Pt 10	0.15	0.000	0.000	0.000
Pt 11	-0.16	0.29	0.000	0.05
Pt 12	*	*	0.000	*
Pt 13	-0.05	0.03	0.000	0.46
Pt 14	-0.07	0.02	0.000	0.10
Pt 15	0.000	0.02	0.000	-0.50
Pt 17	-0.037	*	*	0.18
Pt 19	0.09	-0.18	*	0.000
Pt 20	-0.004	0.06	*	-0.06
Pt 21	-0.03	0.000	*	0.000
Pt 22	-0.01	0.01	*	0.05
Pt 23	0.16	0.03	*	0.03
Pt 25	0.07	0.20	*	-0.26
Pt 26	0.000	-0.04	*	0.000
Mean	-0.005	0.04	-0.003	0.01
SD	0.08	0.12	0.005	0.19
Median	-0.01	0.01	0.001	0.001
25th percentile	-0.05	-0.003	-0.007	-0.01
75th percentile	0.000	0.09	0.001	0.05
p values from one-sample t-test	0.79	0.17	0.17	0.80

*No baseline or longitudinal values recorded

b) Slopes of change over time in videoed cognitive and motor scores

	Cognitive impairment	Extrapyramidal impairment	Pyramidal impairment	Cerebellar impairment
Pt 1	0.023	0.03	0.000	0.000
Pt 5	0.000	0.000	0.000	0.01
Pt 6	-0.014	0.000	0.000	0.02
Pt 7	0.000	0.007	-0.01	-0.02
Pt 8	0.000	-0.03	-0.006	0.05
Pt 9	0.000	0.000	-0.02	0.000
Pt 10	0.000	0.000	0.000	0.000
Pt 11	0.006	0.006	0.000	0.002
Pt 12	0.000	0.000	0.000	0.000
Pt 13	0.000	-0.02	-0.02	0.006
Pt 14	0.000	0.000	-0.04	-0.008
Pt 15	-0.001	0.000	0.000	-0.008

Pt 17	0.000	0.03	0.02	0.007
Pt 19	-0.02	-0.03	-0.07	0.05
Pt 20	0.004	0.000	-0.005	-0.006
Pt 21	-0.008	0.006	0.000	0.04
Pt 22	0.006	0.000	0.006	0.01
Pt 23	0.000	0.000	0.000	0.000
Pt 25	0.000	0.000	0.07	0.000
Pt 26	-0.02	0.000	0.000	-0.02
Mean	-0.001	-0.001	-0.004	0.007
SD	0.009	0.01	0.02	0.02
Median	0.001	0.001	0.001	0.001
25th percentile	-0.001	0.001	-0.01	-0.005
75th percentile	0.001	0.005	0.001	0.01
p values from one-sample t-test	0.60	0.92	0.46	0.14

Appendix V: Associations between decline in MT measures and decline in clinical scores

a) Decline in MT measures versus decline in MMSE

Decline MMSE	Decline MT measures	p value	Slope (B value)
	a) Whole brain histogram analysis		
	AVMTR	0.85	0.01
	PH	0.35	0.46
	PL	0.32	0.09
	MTR25%	0.66	0.01
	MTR50%	0.62	0.01
	MTR75%	0.53*	-0.06
	b) White matter histogram analysis		
	AVMTR	0.62*	-0.04
	PH	0.87	0.22
	PL	0.89	0.02
	MTR25%	0.86	0.03
	MTR50%	0.37*	-0.07
	MTR75%	0.28*	-0.08
	c) Grey matter histogram analysis		
	AVMTR	0.62*	-0.03
	PH	0.70	0.16
	PL	0.58*	-0.06
	MTR25%	0.63*	-0.05
	MTR50%	0.24*	-0.09
	MTR75%	0.98*	-0.002
	d) ROI analysis		
	Right caudate	0.43*	-0.19
	Left caudate	0.65*	-0.11
	Right putamen	0.29	0.15
	Left putamen	0.07*	-0.20
	Right pulvinar	0.72*	-0.04
	Left pulvinar	0.20*	-0.15

*Associations in the unexpected direction

b) Decline in MT measures versus decline in Barthel ADL

Decline ADL	Decline MT measures	p value	Slopes (B value)
	a) Whole brain histogram analysis		
	AVMTR	0.83*	-0.02
	PH	0.74*	-0.18
	PL	0.23*	-0.12
	MTR25%	0.77*	-0.04
	MTR50%	0.16*	-0.13
	MTR75%	0.15	0.13
	b) White matter histogram analysis		
	AVMTR	0.50*	-0.06
	PH	0.40*	-1.30
	PL	0.43*	-0.10
	MTR25%	0.38*	-0.16
	MTR50%	0.10*	0.00

	MTR75%	0.56	0.05
	c) Grey matter histogram analysis		
	AVMTR	0.74	0.03
	PH	0.57*	-0.29
	PL	0.60*	-0.07
	MTR25%	0.74	0.03
	MTR50%	0.49	0.06
	MTR75%	0.63	0.05
	d) ROI analysis		
	Right caudate	0.89	0.04
	Left caudate	0.17	0.34
	Right putamen	0.43*	-0.14
	Left putamen	0.52*	-0.09
	Right pulvinar	0.18	0.17
	Left pulvinar	0.20*	-0.15

*Associations in the unexpected direction

c) Decline in MT measures versus decline in ADAS-COG

Decline ADAS-COG	Decline MT measures	p value	Slope (B value)
	a) Whole brain histogram analysis		
	AVMTR	0.10*	0.00
	PH	0.24	-0.36
	PL	0.89*	0.009
	MTR25%	0.08	-0.10
	MTR50%	0.50	-0.04
	MTR75%	0.13*	0.09
	b) White matter histogram analysis		
	AVMTR	0.60*	0.03
	PH	0.66	-0.38
	PL	0.60*	0.04
	MTR25%	0.82*	0.02
	MTR50%	0.57*	0.03
	MTR75%	0.07*	0.09
	c) Grey matter histogram analysis		
	AVMTR	0.25*	0.05
	PH	0.66	-0.12
	PL	0.22	-0.09
	MTR25%	0.39*	0.05
	MTR50%	0.06*	0.09
	MTR75%	0.35*	0.05
	d) ROI analysis		
	Right caudate	0.65	-0.07
	Left caudate	0.54*	0.10
	Right putamen	0.70	-0.04
	Left putamen	0.16*	0.10
	Right pulvinar	0.11*	0.12
	Left pulvinar	0.06*	0.14

*Associations in the unexpected direction

d) Decline in MT measures versus decline in CGIS

Decline CGIS	Decline MT measures	p value	Slope (B value)
	a) Whole brain histogram analysis		
	AVMTR	0.54	-0.34
	PH	0.27	-3.96
	PL	0.48	-0.46
	MTR25%	0.54	-0.54
	MTR50%	0.16	-0.84
	MTR75%	0.76	-0.20
	b) White matter histogram analysis		
	AVMTR	0.72	-0.20
	PH	0.40	-8.43
	PL	0.55	-0.52
	MTR25%	0.38	-1.07
	MTR50%	0.48	-0.36
	MTR75%	0.85*	0.11
	c) Grey matter histogram analysis		
	AVMTR	0.92	-0.06
	PH	0.73	-1.20
	PL	0.94*	0.08
	MTR25%	0.81	-0.16
	MTR50%	0.87*	0.11
	MTR75%	0.65	-0.30
	d) ROI analysis		
	Right caudate	0.84	-0.41
	Left caudate	0.88*	0.26
	Right putamen	0.54	-0.71
	Left putamen	0.36*	0.86
	Right pulvinar	0.78*	0.23
	Left pulvinar	0.25*	1.01

*Associations in the unexpected direction

e) Decline in MT measures versus decline in Rankin

Decline Rankin	Decline MT measures	p value	Slope (B value)
	a) Whole brain histogram analysis		
	AVMTR	0.44*	0.54
	PH	0.33*	4.45
	PL	0.42*	0.67
	MTR25%	0.70*	0.44
	MTR50%	0.94	-0.06
	MTR75%	0.72*	0.29
	b) White matter histogram analysis		
	AVMTR	0.93*	0.06
	PH	0.91	-1.45
	PL	0.65	-0.51
	MTR25%	0.35	-1.44
	MTR50%	0.99	-0.004
	MTR75%	0.99	-0.006

	c) Grey matter histogram analysis		
	AVMTR	0.40*	0.58
	PH	0.53*	2.75
	PL	0.11*	1.84
	MTR25%	0.82*	0.19
	MTR50%	0.59*	0.41
	MTR75%	0.46*	0.61
	d) ROI analysis		
	Right caudate	0.08*	4.32
	Left caudate	0.17*	2.93
	Right putamen	0.46*	1.11
	Left putamen	0.28*	1.29
	Right pulvinar	0.67	-0.46
	Left pulvinar	0.88	-0.17

*Associations in the unexpected direction

f) Decline in MT measures versus decline in BPRS

Decline BPRS	Decline MT measures	p value	Slope (B value)
	a) Whole brain histogram analysis		
	AVMTR	0.31	-0.03
	PH	0.84	-0.04
	PL	0.82	-0.008
	MTR25%	0.61	-0.02
	MTR50%	0.50	-0.02
	MTR75%	0.34	-0.04
	b) White matter histogram analysis		
	AVMTR	0.56	-0.02
	PH	0.95	-0.04
	PL	0.30	-0.05
	MTR25%	0.33	-0.06
	MTR50%	0.73	-0.01
	MTR75%	0.48	-0.02
	c) Grey matter histogram analysis		
	AVMTR	0.38	-0.02
	PH	0.70*	0.06
	PL	0.54*	0.03
	MTR25%	0.38	-0.03
	MTR50%	0.73	-0.01
	MTR75%	0.35	-0.03
	d) ROI analysis		
	Right caudate	0.50*	0.07
	Left caudate	0.40*	0.08
	Right putamen	0.44	-0.04
	Left putamen	0.82*	0.01
	Right pulvinar	0.48	-0.03
	Left pulvinar	0.93*	0.004

*Associations in the unexpected direction

g) Decline in MT measures versus decline in cognitive impairment

Decline cognitive impairment	Decline MT measures	p value	Slope (B value)
	a) Whole brain histogram analysis		
	AVMTR	0.52	-0.40
	PH	0.16	-5.56
	PL	0.46	-0.54
	MTR25%	0.11	-1.54
	MTR50%	0.11	-1.07
	MTR75%	0.38*	0.63
	b) White matter histogram analysis		
	AVMTR	0.64	-0.30
	PH	0.31	-11.50
	PL	0.60	-0.51
	MTR25%	0.41	-1.13
	MTR50%	0.95*	0.04
	MTR75%	0.32*	0.65
	c) Grey matter histogram analysis		
	AVMTR	0.96	-0.03
	PH	0.45	-2.90
	PL	0.54	-0.65
	MTR25%	0.47	-0.52
	MTR50%	0.68*	0.29
	MTR75%	0.94	-0.06
	d) ROI analysis		
	Right caudate	0.73	-0.79
	Left caudate	0.35*	1.79
	Right putamen	0.30	-1.35
	Left putamen	0.37*	0.95
	Right pulvinar	0.40*	0.79
	Left pulvinar	0.11*	1.50

*Associations in the unexpected direction

h) Associations between decline in MT measures versus decline in cerebellar impairment

Decline cerebellar impairment	Decline MT measures	p value	Slope (B value)
	a) Whole brain histogram analysis		
	AVMTR	0.65*	0.13
	PH	0.73	-0.65
	PL	0.67*	0.14
	MTR25%	0.51*	0.29
	MTR50%	0.25*	0.36
	MTR75%	0.50	-0.22
	b) White matter histogram analysis		
	AVMTR	0.83*	0.06
	PH	0.59	-2.80
	PL	0.54*	0.28

	MTR25%	0.70*	0.24
	MTR50%	0.68*	0.11
	MTR75%	0.65	-0.14
	c) Grey matter histogram analysis		
	AVMTR	0.60	-0.14
	PH	0.52	-1.10
	PL	0.67*	0.20
	MTR25%	0.49	-0.23
	MTR50%	0.34	-0.29
	MTR75%	0.91	-0.04
	d) ROI analysis		
	Right caudate	0.71	-0.38
	Left caudate	0.29	-0.93
	Right putamen	0.93*	0.05
	Left putamen	0.60	-0.25
	Right pulvinar	0.14	-0.62
	Left pulvinar	0.13	-0.16

*Associations in the unexpected direction

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